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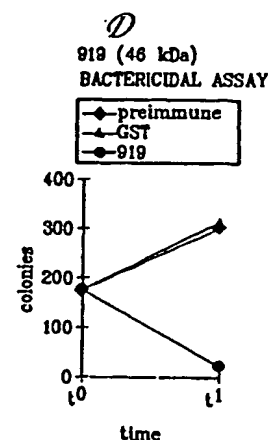
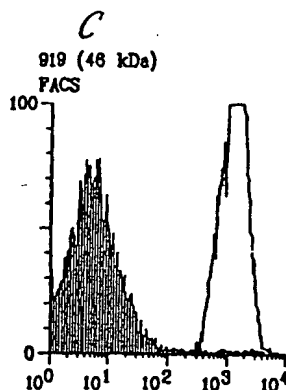
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**A**  
919 (46 kDa)  
PURIFICATION  
MI 919

**B**  
919 (46 kDa)  
WESTERN BLOT  
OMV TP PP



**E**  
919 (46 kDa)  
ELISA assay: positive

(57) Abstract

The invention provides methods of obtaining immunogenic proteins from genomic sequences including *Neisseria*, including the amino acid sequences and the corresponding nucleotide sequences, as well as the genomic sequence of *Neisseria meningitidis B*. The proteins so obtained are useful antigens for vaccines, immunogenic compositions, and/or diagnostics.

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## NEISSERIA GENOMIC SEQUENCES AND METHODS OF THEIR USE

This application claims priority to provisional U.S. application serial no. 60/132,068, filed 30 April 1999; PCT/US99/23573, filed 8 October 1999 (to be published April 2000); and Great Britain application serial no. GB-0004695.3, filed 28 February 2000.

This invention relates to methods of obtaining antigens and immunogens, the antigens and immunogens so obtained, and nucleic acids from the bacterial species: *Neisseria meningitidis*. In particular, it relates to genomic sequences from the bacterium; more particularly its "B" serogroup.

## BACKGROUND

*Neisseria meningitidis* is a non-motile, gram negative diplococcus human pathogen. It colonizes the pharynx, causing meningitis and, occasionally, septicaemia in the absence of meningitis. It is closely related to *N. gonorrhoea*, although one feature that clearly differentiates meningococcus from gonococcus is the presence of a polysaccharide capsule that is present in all pathogenic meningococci.

*N. meningitidis* causes both endemic and epidemic disease. In the United States the attack rate is 0.6-1 per 100,000 persons per year, and it can be much greater during outbreaks. (see Lieberman *et al.* (1996) Safety and Immunogenicity of a Serogroups A/C *Neisseria meningitidis* Oligosaccharide-Protein Conjugate Vaccine in Young Children. *JAMA* 275(19):1499-1503; Schuchat *et al* (1997) Bacterial Meningitis in the United States in 1995. *N Engl J Med* 337(14):970-976). In developing countries, endemic disease rates are much higher and during epidemics incidence rates can reach 500 cases per 100,000 persons per year. Mortality is extremely high, at 10-20% in the United States, and much higher in developing countries. Following the introduction of the conjugate vaccine against *Haemophilus influenzae*, *N. meningitidis* is the major cause of bacterial meningitis at all ages in the United States (Schuchat *et al* (1997) *supra*).

Based on the organism's capsular polysaccharide, 12 serogroups of *N. meningitidis* have been identified. Group A is the pathogen most often implicated in epidemic disease in sub-Saharan Africa. Serogroups B and C are responsible for the vast majority of cases in the



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United States and in most developed countries. Serogroups W135 and Y are responsible for the rest of the cases in the United States and developed countries. The meningococcal vaccine currently in use is a tetravalent polysaccharide vaccine composed of serogroups A, C, Y and W135. Although efficacious in adolescents and adults, it induces a poor immune response and short duration of protection, and cannot be used in infants (e.g., Morbidity and Mortality weekly report, Vol. 46, No. RR-5 (1997)). This is because polysaccharides are T-cell independent antigens that induce a weak immune response that cannot be boosted by repeated immunization. Following the success of the vaccination against *H. influenzae*, conjugate vaccines against serogroups A and C have been developed and are at the final stage of clinical testing (Zollinger WD "New and Improved Vaccines Against Meningococcal Disease". In: *New Generation Vaccines, supra*, pp. 469-488; Lieberman *et al* (1996) *supra*; Costantino *et al* (1992) Development and phase I clinical testing of a conjugate vaccine against meningococcus A (menA) and C (menC) (*Vaccine* 10:691-698)).

Meningococcus B (MenB) remains a problem, however. This serotype currently is responsible for approximately 50% of total meningitis in the United States, Europe, and South America. The polysaccharide approach cannot be used because the MenB capsular polysaccharide is a polymer of  $\alpha(2-8)$ -linked *N*-acetyl neuraminic acid that is also present in mammalian tissue. This results in tolerance to the antigen; indeed, if an immune response were elicited, it would be anti-self, and therefore undesirable. In order to avoid induction of autoimmunity and to induce a protective immune response, the capsular polysaccharide has, for instance, been chemically modified substituting the *N*-acetyl groups with *N*-propionyl groups, leaving the specific antigenicity unaltered (Romero & Outschoorn (1994) Current status of Meningococcal group B vaccine candidates: capsular or non-capsular? *Clin Microbiol Rev* 7(4):559-575).

Alternative approaches to MenB vaccines have used complex mixtures of outer membrane proteins (OMPs), containing either the OMPs alone, or OMPs enriched in porins, or deleted of the class 4 OMPs that are believed to induce antibodies that block bactericidal activity. This approach produces vaccines that are not well characterized. They are able to protect against the homologous strain, but are not effective at large where there are many antigenic variants of the outer membrane proteins. To overcome the antigenic variability, multivalent vaccines containing up to nine different porins have been constructed (e.g.,

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Poolman JT (1992) Development of a meningococcal vaccine. *Infect. Agents Dis.* 4:13-28). Additional proteins to be used in outer membrane vaccines have been the opa and opc proteins, but none of these approaches have been able to overcome the antigenic variability (e.g., Ala'Aldeen & Borriello (1996) The meningococcal transferrin-binding proteins 1 and 2 are both surface exposed and generate bactericidal antibodies capable of killing homologous and heterologous strains. *Vaccine* 14(1):49-53).

A certain amount of sequence data is available for meningococcal and gonococcal genes and proteins (e.g., EP-A-0467714, WO96/29412), but this is by no means complete. The provision of further sequences could provide an opportunity to identify secreted or surface-exposed proteins that are presumed targets for the immune system and which are not antigenically variable or at least are more antigenically conserved than other and more variable regions. Thus, those antigenic sequences that are more highly conserved are preferred sequences. Those sequences specific to *Neisseria meningitidis* or *Neisseria gonorrhoeae* that are more highly conserved are further preferred sequences. For instance, some of the identified proteins could be components of efficacious vaccines against meningococcus B, some could be components of vaccines against all meningococcal serotypes, and others could be components of vaccines against all pathogenic *Neisseriae*. The identification of sequences from the bacterium will also facilitate the production of biological probes, particularly organism-specific probes.

It is thus an object of the invention is to provide Neisserial DNA sequences which (1) encode proteins predicted and/or shown to be antigenic or immunogenic, (2) can be used as probes or amplification primers, and (3) can be analyzed by bioinformatics.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the products of protein expression and purification of the predicted ORF 919 as cloned and expressed in *E. coli*.

Fig. 2 illustrates the products of protein expression and purification of the predicted ORF 279 as cloned and expressed in *E. coli*.

Fig. 3 illustrates the products of protein expression and purification of the predicted ORF 576-1 as cloned and expressed in *E. coli*.

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Fig. 4 illustrates the products of protein expression and purification of the predicted ORF 519-1 as cloned and expressed in *E. coli*.

Fig. 5 illustrates the products of protein expression and purification of the predicted ORF 121-1 as cloned and expressed in *E. coli*.

Fig. 6 illustrates the products of protein expression and purification of the predicted ORF 128-1 as cloned and expressed in *E. coli*.

Fig. 7 illustrates the products of protein expression and purification of the predicted ORF 206 as cloned and expressed in *E. coli*.

Fig. 8 illustrates the products of protein expression and purification of the predicted ORF 287 as cloned and expressed in *E. coli*.

Fig. 9 illustrates the products of protein expression and purification of the predicted ORF 406 as cloned and expressed in *E. coli*.

Fig. 10 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 919 as cloned and expressed in *E. coli*.

Fig. 11 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 279 as cloned and expressed in *E. coli*.

Fig. 12 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 576-1 as cloned and expressed in *E. coli*.

Fig. 13 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 519-1 as cloned and expressed in *E. coli*.

Fig. 14 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 121-1 as cloned and expressed in *E. coli*.

Fig. 15 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 128-1 as cloned and expressed in *E. coli*.

Fig. 16 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 206 as cloned and expressed in *E. coli*.

Fig. 17 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 287 as cloned and expressed in *E. coli*.

Fig. 18 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 406 as cloned and expressed in *E. coli*.

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## THE INVENTION

The first complete sequence of the genome of *N. meningitidis* was disclosed as 961 partial contiguous nucleotide sequences, shown as SEQ ID NOs:1-961 of co-owned PCT/US99/23573 (the '573 application), filed 8 October 1999 (to be published April 2000). A single sequence full length genome of *N. meningitidis* was also disclosed as SEQ ID NO. 1068 of the '573 application. The invention is based on a full length genome of *N. meningitidis* which appears as SEQ ID NO. 1 in the present application as Appendix A hereto. The 961 sequences of the '573 application represent substantially the whole genome of serotype B of *N. meningitidis* (>99.98%). There is partial overlap between some of the 961 contiguous sequences ("contigs") shown in the 961 sequences, which overlap was used to construct the single full length sequence shown in SEQ ID NO. 1 in Appendix A hereto, using the TIGR Assembler [G.S. Sutton et al., *TIGR Assembler: A New Tool for Assembling Large Shotgun Sequencing Projects*, Genome Science and Technology, 1:9-19 (1995)]. Some of the nucleotides in the contigs had been previously released. (See [ftp://11ftp.tigr.org/pub/data/n\\_meningitidis](ftp://11ftp.tigr.org/pub/data/n_meningitidis) on the world-wide web or "WWW"). The coordinates of the 2508 released sequences in the present contigs are presented in Appendix A of the '573 application. These data include the contig number (or i.d.) as presented in the first column; the name of the sequence as found on WWW is in the second column; with the coordinates of the contigs in the third and fourth columns, respectively. The sequences of certain MenB ORFs presented in Appendix B of the '573 application feature in International Patent Application filed by Chiron SpA on October 9, 1998 (PCT/IB98/01665) and January 14, 1999 (PCT/IB99/00103) respectively. Appendix B hereto provides a listing of 2158 open reading frames contained within the full length sequence found in SEQ ID NO. 1 in Appendix A hereto. The information set forth in Appendix B hereto includes the "NMB" name of the sequence, the putative translation product, and the beginning and ending nucleotide positions within SEQ ID NO. 1 which comprise the open reading frames. These open reading frames are referred to herein as the "NMB open reading frames".

In a first aspect, the invention provides nucleic acid including the *N. meningitidis* nucleotide sequence shown in SEQ ID NO. 1 in Appendix A hereto. It also provides nucleic acid comprising sequences having sequence identity to the nucleotide sequence disclosed herein. Depending on the particular sequence, the degree of sequence identity is preferably

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greater than 50% (e.g., 60%, 70%, 80%, 90%, 95%, 99% or more). These sequences include, for instance, mutants and allelic variants. The degree of sequence identity cited herein is determined across the length of the sequence determined by the Smith-Waterman homology search algorithm as implemented in MPSRCH program (Oxford Molecular) using an affine gap search with the following parameters: gap open penalty 12, gap extension penalty 1.

The invention also provides nucleic acid including a fragment of one or more of the nucleotide sequences set out herein, including the NMB open reading frames shown in Appendix B hereto. The fragment should comprise at least  $n$  consecutive nucleotides from the sequences and, depending on the particular sequence,  $n$  is 10 or more (e.g., 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 60, 75, 100 or more). Preferably, the fragment is unique to the genome of *N. meningitidis*, that is to say it is not present in the genome of another organism. More preferably, the fragment is unique to the genome of strain B of *N. meningitidis*. The invention also provides nucleic acid that hybridizes to those provided herein. Conditions for hybridizing are disclosed herein.

The invention also provides nucleic acid including sequences complementary to those described above (e.g., for antisense, for probes, or for amplification primers).

Nucleic acid according to the invention can, of course, be prepared in many ways (e.g., by chemical synthesis, from DNA libraries, from the organism itself, etc.) and can take various forms (e.g., single-stranded, double-stranded, vectors, probes, primers, etc.). The term "nucleic acid" includes DNA and RNA, and also their analogs, such as those containing modified backbones, and also peptide nucleic acid (PNA) etc.

It will be appreciated that, as SEQ ID NOs:1-961 of the '573 application represent the substantially complete genome of the organism, with partial overlap, references to SEQ ID NOs:1-961 of the '573 application include within their scope references to the complete genomic sequence, that is, SEQ ID NO. 1 hereof. For example, where two SEQ ID NOs overlap, the invention encompasses the single sequence which is formed by assembling the two overlapping sequences, which full sequence will be found in SEQ ID NO. 1 hereof. Thus, for instance, a nucleotide sequence which bridges two SEQ ID NOs but is not present in its entirety in either SEQ ID NO is still within the scope of the invention. Such a sequence will be present in its entirety in the single full length sequence of SEQ ID NO. 1 of the present application.

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The invention also provides vectors including nucleotide sequences of the invention (e.g., expression vectors, sequencing vectors, cloning vectors, etc.) and host cells transformed with such vectors.

According to a further aspect, the invention provides a protein including an amino acid sequence encoded within a *N. meningitidis* nucleotide sequence set out herein. It also provides proteins comprising sequences having sequence identity to those proteins. Depending on the particular sequence, the degree of sequence identity is preferably greater than 50% (e.g., 60%, 70%, 80%, 90%, 95%, 99% or more). Sequence identity is determined as above disclosed. These homologous proteins include mutants and allelic variants, encoded within the *N. meningitidis* nucleotide sequence set out herein.

The invention further provides proteins including fragments of an amino acid sequence encoded within a *N. meningitidis* nucleotide sequence set out in the sequence listing. The fragments should comprise at least *n* consecutive amino acids from the sequences and, depending on the particular sequence, *n* is 7 or more (e.g., 8, 10, 12, 14, 16, 18, 20 or more). Preferably the fragments comprise an epitope from the sequence.

The proteins of the invention can, of course, be prepared by various means (e.g., recombinant expression, purification from cell culture, chemical synthesis, etc.) and in various forms (e.g. native, fusions etc.). They are preferably prepared in substantially isolated form (i.e., substantially free from other *N. meningitidis* host cell proteins).

Various tests can be used to assess the *in vivo* immunogenicity of the proteins of the invention. For example, the proteins can be expressed recombinantly or chemically synthesized and used to screen patient sera by immunoblot. A positive reaction between the protein and patient serum indicates that the patient has previously mounted an immune response to the protein in question; i.e., the protein is an immunogen. This method can also be used to identify immunodominant proteins.

The invention also provides nucleic acid encoding a protein of the invention.

In a further aspect, the invention provides a computer, a computer memory, a computer storage medium (e.g., floppy disk, fixed disk, CD-ROM, etc.), and/or a computer database containing the nucleotide sequence of nucleic acid according to the invention. Preferably, it contains one or more of the *N. meningitidis* nucleotide sequences set out herein.

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This may be used in the analysis of the *N. meningitidis* nucleotide sequences set out herein. For instance, it may be used in a search to identify open reading frames (ORFs) or coding sequences within the sequences.

In a further aspect, the invention provides a method for identifying an amino acid sequence, comprising the step of searching for putative open reading frames or protein-coding sequences within a *N. meningitidis* nucleotide sequence set out herein. Similarly, the invention provides the use of a *N. meningitidis* nucleotide sequence set out herein in a search for putative open reading frames or protein-coding sequences.

Open-reading frame or protein-coding sequence analysis is generally performed on a computer using standard bioinformatic techniques. Typical algorithms or program used in the analysis include ORFFINDER (NCBI), GENMARK [Borodovsky & McIninch (1993) *Computers Chem* 17:122-133], and GLIMMER [Salzberg et al. (1998) *Nucl Acids Res* 26:544-548].

A search for an open reading frame or protein-coding sequence may comprise the steps of searching a *N. meningitidis* nucleotide sequence set out herein for an initiation codon and searching the upstream sequence for an in-frame termination codon. The intervening codons represent a putative protein-coding sequence. Typically, all six possible reading frames of a sequence will be searched.

An amino acid sequence identified in this way can be expressed using any suitable system to give a protein. This protein can be used to raise antibodies which recognize epitopes within the identified amino acid sequence. These antibodies can be used to screen *N. meningitidis* to detect the presence of a protein comprising the identified amino acid sequence.

Furthermore, once an ORF or protein-coding sequence is identified, the sequence can be compared with sequence databases. Sequence analysis tools can be found at NCBI (<http://www.ncbi.nlm.nih.gov>) e.g., the algorithms BLAST, BLAST2, BLASTn, BLASTp, tBLASTn, BLASTx, & tBLASTx [see also Altschul *et al.* (1997) Gapped BLAST and PSI-BLAST: new generation of protein database search programs. *Nucleic Acids Research* 25:2289-3402]. Suitable databases for comparison include the nonredundant GenBank, EMBL, DDBJ and PDB sequences, and the nonredundant GenBank CDS translations, PDB,

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SwissProt, Spupdate and PIR sequences. This comparison may give an indication of the function of a protein.

Hydrophobic domains in an amino acid sequence can be predicted using algorithms such as those based on the statistical studies of Esposti *et al.* [Critical evaluation of the hydropathy of membrane proteins (1990) *Eur J Biochem* 190:207-219]. Hydrophobic domains represent potential transmembrane regions or hydrophobic leader sequences, which suggest that the proteins may be secreted or be surface-located. These properties are typically representative of good immunogens.

Similarly, transmembrane domains or leader sequences can be predicted using the PSORT algorithm (<http://www.psort.nibb.ac.jp>), and functional domains can be predicted using the MOTIFS program (GCG Wisconsin & PROSITE).

The invention also provides nucleic acid including an open reading frame or protein-coding sequence present in a *N. meningitidis* nucleotide sequence set out herein. Furthermore, the invention provides a protein including the amino acid sequence encoded by this open reading frame or protein-coding sequence.

According to a further aspect, the invention provides antibodies which bind to these proteins. These may be polyclonal or monoclonal and may be produced by any suitable means known to those skilled in the art.

The antibodies of the invention can be used in a variety of ways, e.g., for confirmation that a protein is expressed, or to confirm where a protein is expressed. Labeled antibody (e.g., fluorescent labeling for FACS) can be incubated with intact bacteria and the presence of label on the bacterial surface confirms the location of the protein, for instance.

According to a further aspect, the invention provides compositions including protein, antibody, and/or nucleic acid according to the invention. These compositions may be suitable as vaccines, as immunogenic compositions, or as diagnostic reagents.

The invention also provides nucleic acid, protein, or antibody according to the invention for use as medicaments (e.g., as vaccines) or as diagnostic reagents. It also provides the use of nucleic acid, protein, or antibody according to the invention in the manufacture of (i) a medicament for treating or preventing infection due to Neisserial bacteria (ii) a diagnostic reagent for detecting the presence of Neisserial bacteria or of antibodies raised against Neisserial bacteria. Said Neisserial bacteria may be any species or



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strain (such as *N. gonorrhoeae*) but are preferably *N. meningitidis*, especially strain A, strain B or strain C.

In still yet another aspect, the present invention provides for compositions including proteins, nucleic acid molecules, or antibodies. More preferable aspects of the present invention are drawn to immunogenic compositions of proteins. Further preferable aspects of the present invention contemplate pharmaceutical immunogenic compositions of proteins or vaccines and the use thereof in the manufacture of a medicament for the treatment or prevention of infection due to Neisserial bacteria, preferably infection of MenB.

The invention also provides a method of treating a patient, comprising administering to the patient a therapeutically effective amount of nucleic acid, protein, and/or antibody according to the invention.

According to further aspects, the invention provides various processes.

A process for producing proteins of the invention is provided, comprising the step of culturing a host cell according to the invention under conditions which induce protein expression. A process which may further include chemical synthesis of proteins and/or chemical synthesis (at least in part) of nucleotides.

A process for detecting polynucleotides of the invention is provided, comprising the steps of: (a) contacting a nucleic probe according to the invention with a biological sample under hybridizing conditions to form duplexes; and (b) detecting said duplexes.

A process for detecting proteins of the invention is provided, comprising the steps of: (a) contacting an antibody according to the invention with a biological sample under conditions suitable for the formation of an antibody-antigen complexes; and (b) detecting said complexes.

Another aspect of the present invention provides for a process for detecting antibodies that selectably bind to antigens or polypeptides or proteins specific to any species or strain of Neisserial bacteria and preferably to strains of *N. gonorrhoeae* but more preferably to strains of *N. meningitidis*, especially strain A, strain B or strain C, more preferably MenB, where the process comprises the steps of: (a) contacting antigen or polypeptide or protein according to the invention with a biological sample under conditions suitable for the formation of an antibody-antigen complexes; and (b) detecting said complexes.

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Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

#### Methodology - Summary of standard procedures and techniques.

##### General

This invention provides *Neisseria meningitidis* MenB nucleotide sequences, amino acid sequences encoded therein. With these disclosed sequences, nucleic acid probe assays and expression cassettes and vectors can be produced. The proteins can also be chemically synthesized. The expression vectors can be transformed into host cells to produce proteins. The purified or isolated polypeptides can be used to produce antibodies to detect MenB proteins. Also, the host cells or extracts can be utilized for biological assays to isolate agonists or antagonists. In addition, with these sequences one can search to identify open reading frames and identify amino acid sequences. The proteins may also be used in immunogenic compositions and as vaccine components.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature e.g., *Sambrook Molecular Cloning; A Laboratory Manual, Second Edition* (1989); *DNA Cloning, Volumes I and ii* (D.N Glover ed. 1985); *Oligonucleotide Synthesis* (M.J. Gait ed, 1984); *Nucleic Acid Hybridization* (B.D. Hames & S.J. Higgins eds. 1984); *Transcription and Translation* (B.D. Hames & S.J. Higgins eds. 1984); *Animal Cell Culture* (R.I. Freshney ed. 1986); *Immobilized Cells and Enzymes* (IRL Press, 1986); B. Perbal, *A Practical Guide to Molecular Cloning* (1984); the *Methods in Enzymology* series (Academic Press, Inc.), especially volumes 154 & 155; *Gene Transfer Vectors for Mammalian Cells* (J.H. Miller and M.P. Calos eds. 1987, Cold Spring Harbor Laboratory); Mayer and Walker, eds. (1987), *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London); Scopes, (1987) *Protein Purification: Principles and Practice*, Second Edition (Springer-Verlag, N.Y.), and *Handbook of Experimental Immunology, Volumes I-IV* (D.M. Weir and C.C. Blackwell eds 1986).

Standard abbreviations for nucleotides and amino acids are used in this specification.

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All publications, patents, and patent applications cited herein are incorporated in full by reference.

#### Expression systems

The *Neisseria* MenB nucleotide sequences can be expressed in a variety of different expression systems; for example those used with mammalian cells, plant cells, baculoviruses, bacteria, and yeast.

##### i. Mammalian Systems

Mammalian expression systems are known in the art. A mammalian promoter is any DNA sequence capable of binding mammalian RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g., structural gene) into mRNA. A promoter will have a transcription initiating region, which is usually placed proximal to the 5' end of the coding sequence, and a TATA box, usually located 25-30 base pairs (bp) upstream of the transcription initiation site. The TATA box is thought to direct RNA polymerase II to begin RNA synthesis at the correct site. A mammalian promoter will also contain an upstream promoter element, usually located within 100 to 200 bp upstream of the TATA box. An upstream promoter element determines the rate at which transcription is initiated and can act in either orientation (Sambrook et al. (1989) "Expression of Cloned Genes in Mammalian Cells." In *Molecular Cloning: A Laboratory Manual*, 2nd ed.).

Mammalian viral genes are often highly expressed and have a broad host range; therefore sequences encoding mammalian viral genes provide particularly useful promoter sequences. Examples include the SV40 early promoter, mouse mammary tumor virus LTR promoter, adenovirus major late promoter (Ad MLP), and herpes simplex virus promoter. In addition, sequences derived from non-viral genes, such as the murine metallothionein gene, also provide useful promoter sequences. Expression may be either constitutive or regulated (inducible). Depending on the promoter selected, many promoters may be inducible using known substrates, such as the use of the mouse mammary tumor virus (MMTV) promoter with the glucocorticoid responsive element (GRE) that is induced by glucocorticoid in hormone-responsive transformed cells (see for example, U.S. Patent 5,783,681).

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The presence of an enhancer element (enhancer), combined with the promoter elements described above, will usually increase expression levels. An enhancer is a regulatory DNA sequence that can stimulate transcription up to 1000-fold when linked to homologous or heterologous promoters, with synthesis beginning at the normal RNA start site. Enhancers are also active when they are placed upstream or downstream from the transcription initiation site, in either normal or flipped orientation, or at a distance of more than 1000 nucleotides from the promoter (Maniatis et al. (1987) *Science* 236:1237; Alberts et al. (1989) *Molecular Biology of the Cell*, 2nd ed.). Enhancer elements derived from viruses may be particularly useful, because they usually have a broader host range. Examples include the SV40 early gene enhancer (Dijkema et al (1985) *EMBO J.* 4:761) and the enhancer/promoters derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus (Gorman et al. (1982b) *Proc. Natl. Acad. Sci.* 79:6777) and from human cytomegalovirus (Boshart et al. (1985) *Cell* 41:521). Additionally, some enhancers are regulatable and become active only in the presence of an inducer, such as a hormone or metal ion (Sassone-Corsi and Borelli (1986) *Trends Genet.* 2:215; Maniatis et al. (1987) *Science* 236:1237).

A DNA molecule may be expressed intracellularly in mammalian cells. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein will always be a methionine, which is encoded by the ATG start codon. If desired, the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide.

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in mammalian cells. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can be cleaved either *in vivo* or *in vitro*. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The adenovirus tripartite leader is an example of a leader sequence that provides for secretion of a foreign protein in mammalian cells.

Usually, transcription termination and polyadenylation sequences recognized by mammalian cells are regulatory regions located 3' to the translation stop codon and thus, together with the promoter elements, flank the coding sequence. The 3' terminus of the

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mature mRNA is formed by site-specific post-transcriptional cleavage and polyadenylation (Birnstiel et al. (1985) *Cell* 41:349; Proudfoot and Whitelaw (1988) "Termination and 3' end processing of eukaryotic RNA. In *Transcription and splicing* (ed. B.D. Hames and D.M. Glover); Proudfoot (1989) *Trends Biochem. Sci.* 14:105). These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminator/polyadenylation signals include those derived from SV40 (Sambrook et al (1989) "Expression of cloned genes in cultured mammalian cells." In *Molecular Cloning: A Laboratory Manual*).

Usually, the above-described components, comprising a promoter, polyadenylation signal, and transcription termination sequence are put together into expression constructs. Enhancers, introns with functional splice donor and acceptor sites, and leader sequences may also be included in an expression construct, if desired. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (e.g., plasmids)-capable of stable maintenance in a host, such as mammalian cells or bacteria. Mammalian replication systems include those derived from animal viruses, which require trans-acting factors to replicate. For example, plasmids containing the replication systems of papovaviruses, such as SV40 (Gluzman (1981) *Cell* 23:175) or polyomavirus, replicate to extremely high copy number in the presence of the appropriate viral T antigen. Additional examples of mammalian replicons include those derived from bovine papillomavirus and Epstein-Barr virus. Additionally, the replicon may have two replication systems, thus allowing it to be maintained, for example, in mammalian cells for expression and in a prokaryotic host for cloning and amplification. Examples of such mammalian-bacteria shuttle vectors include pMT2 (Kaufman et al. (1989) *Mol. Cell. Biol.* 9:946) and pHEBO (Shimizu et al. (1986) *Mol. Cell. Biol.* 6:1074).

The transformation procedure used depends upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection

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(ATCC), including but not limited to, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and a number of other cell lines.

ii. Plant Cellular Expression Systems

There are many plant cell culture and whole plant genetic expression systems known in the art. Exemplary plant cellular genetic expression systems include those described in patents, such as: U.S. 5,693,506; US 5,659,122; and US 5,608,143. Additional examples of genetic expression in plant cell culture has been described by Zenk, *Phytochemistry* 30:3861-3863 (1991). Descriptions of plant protein signal peptides may be found in addition to the references described above in Vaultcombe et al., *Mol. Gen. Genet.* 209:33-40 (1987); Chandler et al., *Plant Molecular Biology* 3:407-418 (1984); Rogers, *J. Biol. Chem.* 260:3731-3738 (1985); Rothstein et al., *Gene* 55:353-356 (1987); Whittier et al., *Nucleic Acids Research* 15:2515-2535 (1987); Wirsal et al., *Molecular Microbiology* 3:3-14 (1989); Yu et al., *Gene* 122:247-253 (1992). A description of the regulation of plant gene expression by the phytohormone, gibberellic acid and secreted enzymes induced by gibberellic acid can be found in R.L. Jones and J. MacMillin, Gibberellins: in: *Advanced Plant Physiology*, Malcolm B. Wilkins, ed., 1984 Pitman Publishing Limited, London, pp. 21-52. References that describe other metabolically-regulated genes: Sheen, *Plant Cell*, 2:1027-1038(1990); Maas et al., *EMBO J.* 9:3447-3452 (1990); Benkel and Hickey, *Proc. Natl. Acad. Sci.* 84:1337-1339 (1987)

Typically, using techniques known in the art, a desired polynucleotide sequence is inserted into an expression cassette comprising genetic regulatory elements designed for operation in plants. The expression cassette is inserted into a desired expression vector with companion sequences upstream and downstream from the expression cassette suitable for expression in a plant host. The companion sequences will be of plasmid or viral origin and provide necessary characteristics to the vector to permit the vectors to move DNA from an original cloning host, such as bacteria, to the desired plant host. The basic bacterial/plant vector construct will preferably provide a broad host range prokaryote replication origin; a prokaryote selectable marker; and, for *Agrobacterium* transformations, T DNA sequences for *Agrobacterium*-mediated transfer to plant chromosomes. Where the heterologous gen is not

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readily amenable to detection, the construct will preferably also have a selectable marker gene suitable for determining if a plant cell has been transformed. A general review of suitable markers, for example for the members of the grass family, is found in Wilkink and Dons, 1993, *Plant Mol. Biol. Repr.*, 11(2):165-185.

Sequences suitable for permitting integration of the heterologous sequence into the plant genome are also recommended. These might include transposon sequences and the like for homologous recombination as well as Ti sequences which permit random insertion of a heterologous expression cassette into a plant genome. Suitable prokaryote selectable markers include resistance toward antibiotics such as ampicillin or tetracycline. Other DNA sequences encoding additional functions may also be present in the vector, as is known in the art.

The nucleic acid molecules of the subject invention may be included into an expression cassette for expression of the protein(s) of interest. Usually, there will be only one expression cassette, although two or more are feasible. The recombinant expression cassette will contain in addition to the heterologous protein encoding sequence the following elements, a promoter region, plant 5' untranslated sequences, initiation codon depending upon whether or not the structural gene comes equipped with one, and a transcription and translation termination sequence. Unique restriction enzyme sites at the 5' and 3' ends of the cassette allow for easy insertion into a pre-existing vector.

A heterologous coding sequence may be for any protein relating to the present invention. The sequence encoding the protein of interest will encode a signal peptide which allows processing and translocation of the protein, as appropriate, and will usually lack any sequence which might result in the binding of the desired protein of the invention to a membrane. Since, for the most part, the transcriptional initiation region will be for a gene which is expressed and translocated during germination, by employing the signal peptide which provides for translocation, one may also provide for translocation of the protein of interest. In this way, the protein(s) of interest will be translocated from the cells in which they are expressed and may be efficiently harvested. Typically secretion in seeds are across the aleurone or scutellar epithelium layer into the endosperm of the seed. While it is not required that the protein be secreted from the cells in which the protein is produced, this facilitates the isolation and purification of the recombinant protein.

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Since the ultimate expression of the desired gene product will be in a eucaryotic cell it is desirable to determine whether any portion of the cloned gene contains sequences which will be processed out as introns by the host's splicosome machinery. If so, site-directed mutagenesis of the "intron" region may be conducted to prevent losing a portion of the genetic message as a false intron code, Reed and Maniatis, *Cell* 41:95-105, 1985.

The vector can be microinjected directly into plant cells by use of micropipettes to mechanically transfer the recombinant DNA. Crossway, *Mol. Gen. Genet.*, 202:179-185, 1985. The genetic material may also be transferred into the plant cell by using polyethylene glycol, Krens, et al., *Nature*, 296, 72-74, 1982. Another method of introduction of nucleic acid segments is high velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface, Klein, et al., *Nature*, 327, 70-73, 1987 and Knudsen and Muller, 1991, *Planta*, 185:330-336 teaching particle bombardment of barley endosperm to create transgenic barley. Yet another method of introduction would be fusion of protoplasts with other entities, either minicells, cells, lysosomes or other fusible lipid-surfaced bodies, Fraley, et al., *Proc. Natl. Acad. Sci. USA*, 79, 1859-1863, 1982.

The vector may also be introduced into the plant cells by electroporation. (Fromm et al., *Proc. Natl. Acad. Sci. USA* 82:5824, 1985). In this technique, plant protoplasts are electroporated in the presence of plasmids containing the gene construct. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and form plant callus.

All plants from which protoplasts can be isolated and cultured to give whole regenerated plants can be transformed by the present invention so that whole plants are recovered which contain the transferred gene. It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to all major species of sugarcane, sugar beet, cotton, fruit and other trees, legumes and vegetables. Some suitable plants include, for example, species from the genera *Fragaria*, *Lotus*, *Medicago*, *Onobrychis*, *Trifolium*, *Trigonella*, *Vigna*, *Citrus*, *Linum*, *Geranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*, *Raphanus*, *Sinapis*, *Atropa*, *Capsicum*, *Datura*, *Hyoscyamus*, *Lycopersion*, *Nicotiana*, *Solanum*, *Petunia*, *Digitalis*, *Majorana*, *Cichorium*, *Helianthus*, *Lactuca*, *Bromus*, *Asparagus*, *Antirrhinum*, *Hererocallis*, *Nemesia*, *Pelargonium*, *Panicum*, *Pennisetum*,



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*Ranunculus, Senecio, Salpiglossis, Cucumis, Browaalia, Glycine, Lolium, Zea, Triticum, Sorghum, and Datura.*

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts containing copies of the heterologous gene is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced from the protoplast suspension. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Shoots and roots normally develop simultaneously. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is fully reproducible and repeatable.

In some plant cell culture systems, the desired protein of the invention may be excreted or alternatively, the protein may be extracted from the whole plant. Where the desired protein of the invention is secreted into the medium, it may be collected. Alternatively, the embryos and embryoless-half seeds or other plant tissue may be mechanically disrupted to release any secreted protein between cells and tissues. The mixture may be suspended in a buffer solution to retrieve soluble proteins. Conventional protein isolation and purification methods will be then used to purify the recombinant protein. Parameters of time, temperature pH, oxygen, and volumes will be adjusted through routine methods to optimize expression and recovery of heterologous protein.

### iii. Baculovirus Systems

The polynucleotide encoding the protein can also be inserted into a suitable insect expression vector, and is operably linked to the control elements within that vector. Vector construction employs techniques which are known in the art. Generally, the components of the expression system include a transfer vector, usually a bacterial plasmid, which contains both a fragment of the baculovirus genome, and a convenient restriction site for insertion of the heterologous gene or genes to be expressed; a wild type baculovirus with a sequence homologous to the baculovirus-specific fragment in the transfer vector (this allows for the

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homologous recombination of the heterologous gene in to the baculovirus genome); and appropriate insect host cells and growth media.

After inserting the DNA sequence encoding the protein into the transfer vector, the vector and the wild type viral genome are transfected into an insect host cell where the vector and viral genome are allowed to recombine. The packaged recombinant virus is expressed and recombinant plaques are identified and purified. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *inter alia*, Invitrogen, San Diego CA ("MaxBac" kit). These techniques are generally known to those skilled in the art and fully described in Summers and Smith, *Texas Agricultural Experiment Station Bulletin No. 1555* (1987) (hereinafter "Summers and Smith").

Prior to inserting the DNA sequence encoding the protein into the baculovirus genome, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are usually assembled into an intermediate transplacement construct (transfer vector). This construct may contain a single gene and operably linked regulatory elements; multiple genes, each with its own set of operably linked regulatory elements; or multiple genes, regulated by the same set of regulatory elements. Intermediate transplacement constructs are often maintained in a replicon, such as an extrachromosomal element (e.g., plasmids) capable of stable maintenance in a host, such as a bacterium. The replicon will have a replication system, thus allowing it to be maintained in a suitable host for cloning and amplification.

Currently, the most commonly used transfer vector for introducing foreign genes into AcNPV is pAc373. Many other vectors, known to those of skill in the art, have also been designed. These include, for example, pVL985 (which alters the polyhedrin start codon from ATG to ATT, and which introduces a BamHI cloning site 32 basepairs downstream from the ATT; see Luckow and Summers, *Virology* (1989) 17:31.

The plasmid usually also contains the polyhedrin polyadenylation signal (Miller et al. (1988) *Ann. Rev. Microbiol.*, 42:177) and a prokaryotic ampicillin-resistance (*amp*) gene and origin of replication for selection and propagation in *E. coli*.

Baculovirus transfer vectors usually contain a baculovirus promoter. A baculovirus promoter is any DNA sequence capable of binding a baculovirus RNA polymerase and initiating the downstream (5' to 3') transcription of a coding sequence (e.g., structural gene)

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into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A baculovirus transfer vector may also have a second domain called an enhancer, which, if present, is usually distal to the structural gene. Expression may be either regulated or constitutive.

Structural genes, abundantly transcribed at late times in a viral infection cycle, provide particularly useful promoter sequences. Examples include sequences derived from the gene encoding the viral polyhedron protein, Friesen et al., (1986) "The Regulation of Baculovirus Gene Expression," in: *The Molecular Biology of Baculoviruses* (ed. Walter Doerfler); EPO Publ. Nos. 127 839 and 155 476; and the gene encoding the p10 protein, Vlak et al., (1988), *J. Gen. Virol.* 69:765.

DNA encoding suitable signal sequences can be derived from genes for secreted insect or baculovirus proteins, such as the baculovirus polyhedrin gene (Carbonell et al. (1988) *Gene*, 73:409). Alternatively, since the signals for mammalian cell posttranslational modifications (such as signal peptide cleavage, proteolytic cleavage, and phosphorylation) appear to be recognized by insect cells, and the signals required for secretion and nuclear accumulation also appear to be conserved between the invertebrate cells and vertebrate cells, leaders of non-insect origin, such as those derived from genes encoding human (alpha)  $\alpha$ -interferon, Maeda et al., (1985), *Nature* 315:592; human gastrin-releasing peptide, Lebacqz-Verheyden et al., (1988), *Molec. Cell. Biol.* 8:3129; human IL-2, Smith et al., (1985) *Proc. Nat'l Acad. Sci. USA*, 82:8404; mouse IL-3, (Miyajima et al., (1987) *Gene* 58:273; and human glucocerebrosidase, Martin et al. (1988) *DNA*, 7:99, can also be used to provide for secretion in insects.

A recombinant polypeptide or polyprotein may be expressed intracellularly or, if it is expressed with the proper regulatory sequences, it can be secreted. Good intracellular expression of nonfused foreign proteins usually requires heterologous genes that ideally have a short leader sequence containing suitable translation initiation signals preceding an ATG start signal. If desired, methionine at the N-terminus may be cleaved from the mature protein by *in vitro* incubation with cyanogen bromide.

Alternatively, recombinant polyproteins or proteins which are not naturally secreted can be secreted from the insect cell by creating chimeric DNA molecules that encode a fusion

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protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in insects. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the translocation of the protein into the endoplasmic reticulum.

After insertion of the DNA sequence and/or the gene encoding the expression product precursor of the protein, an insect cell host is co-transformed with the heterologous DNA of the transfer vector and the genomic DNA of wild type baculovirus -- usually by co-transfection. The promoter and transcription termination sequence of the construct will usually comprise a 2-5kb section of the baculovirus genome. Methods for introducing heterologous DNA into the desired site in the baculovirus virus are known in the art. (See Summers and Smith *supra*; Ju et al. (1987); Smith et al., *Mol. Cell. Biol.* (1983) 3:2156; and Luckow and Summers (1989)). For example, the insertion can be into a gene such as the polyhedrin gene, by homologous double crossover recombination; insertion can also be into a restriction enzyme site engineered into the desired baculovirus gene. Miller et al., (1989), *Bioessays* 4:91. The DNA sequence, when cloned in place of the polyhedrin gene in the expression vector, is flanked both 5' and 3' by polyhedrin-specific sequences and is positioned downstream of the polyhedrin promoter.

The newly formed baculovirus expression vector is subsequently packaged into an infectious recombinant baculovirus. Homologous recombination occurs at low frequency (between about 1% and about 5%); thus, the majority of the virus produced after cotransfection is still wild-type virus. Therefore, a method is necessary to identify recombinant viruses. An advantage of the expression system is a visual screen allowing recombinant viruses to be distinguished. The polyhedrin protein, which is produced by the native virus, is produced at very high levels in the nuclei of infected cells at late times after viral infection. Accumulated polyhedrin protein forms occlusion bodies that also contain embedded particles. These occlusion bodies, up to 15  $\mu$ m in size, are highly refractile, giving them a bright shiny appearance that is readily visualized under the light microscope. Cells infected with recombinant viruses lack occlusion bodies. To distinguish recombinant virus from wild-type virus, the transfection supernatant is plaqued onto a monolayer of insect cells by techniques known to those skilled in the art. Namely, the plaques are screened under the light microscope for the presence (indicative of wild-type virus) or absence (indicative of

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recombinant virus) of occlusion bodies. *Current Protocols in Microbiology* Vol. 2 (Ausubel et al. eds) at 16.8 (Supp. 10, 1990); Summers and Smith, *supra*; Miller et al. (1989).

Recombinant baculovirus expression vectors have been developed for infection into several insect cells. For example, recombinant baculoviruses have been developed for, *inter alia*: *Aedes aegypti*, *Autographa californica*, *Bombyx mori*, *Drosophila melanogaster*, *Spodoptera frugiperda*, and *Trichoplusia ni* (PCT Pub. No. WO 89/046699; Carbonell et al., (1985) *J. Virol.* 56:153; Wright (1986) *Nature* 321:718; Smith et al., (1983) *Mol. Cell. Biol.* 3:2156; and see generally, Fraser, et al. (1989) *In Vitro Cell. Dev. Biol.* 25:225).

Cells and cell culture media are commercially available for both direct and fusion expression of heterologous polypeptides in a baculovirus/expression system; cell culture technology is generally known to those skilled in the art. See, e.g., Summers and Smith *supra*.

The modified insect cells may then be grown in an appropriate nutrient medium, which allows for stable maintenance of the plasmid(s) present in the modified insect host. Where the expression product gene is under inducible control, the host may be grown to high density, and expression induced. Alternatively, where expression is constitutive, the product will be continuously expressed into the medium and the nutrient medium must be continuously circulated, while removing the product of interest and augmenting depleted nutrients. The product may be purified by such techniques as chromatography, e.g., HPLC, affinity chromatography, ion exchange chromatography, etc.; electrophoresis; density gradient centrifugation; solvent extraction, or the like. As appropriate, the product may be further purified, as required, so as to remove substantially any insect proteins which are also secreted in the medium or result from lysis of insect cells, so as to provide a product which is at least substantially free of host debris, e.g., proteins, lipids and polysaccharides.

In order to obtain protein expression, recombinant host cells derived from the transformants are incubated under conditions which allow expression of the recombinant protein encoding sequence. These conditions will vary, dependent upon the host cell selected. However, the conditions are readily ascertainable to those of ordinary skill in the art, based upon what is known in the art.

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## iv. Bacterial Systems

Bacterial expression techniques are known in the art. A bacterial promoter is any DNA sequence capable of binding bacterial RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g. structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A bacterial promoter may also have a second domain called an operator, that may overlap an adjacent RNA polymerase binding site at which RNA synthesis begins. The operator permits negative regulated (inducible) transcription, as a gene repressor protein may bind the operator and thereby inhibit transcription of a specific gene. Constitutive expression may occur in the absence of negative regulatory elements, such as the operator. In addition, positive regulation may be achieved by a gene activator protein binding sequence, which, if present is usually proximal (5') to the RNA polymerase binding sequence. An example of a gene activator protein is the catabolite activator protein (CAP), which helps initiate transcription of the lac operon in *Escherichia coli* (*E. coli*) (Raibaud *et al.* (1984) *Annu. Rev. Genet.* 18:173). Regulated expression may therefore be either positive or negative, thereby either enhancing or reducing transcription.

Sequences encoding metabolic pathway enzymes provide particularly useful promoter sequences. Examples include promoter sequences derived from sugar metabolizing enzymes, such as galactose, lactose (*lac*) (Chang *et al.* (1977) *Nature* 198:1056), and maltose. Additional examples include promoter sequences derived from biosynthetic enzymes such as tryptophan (*trp*) (Goeddel *et al.* (1980) *Nuc. Acids Res.* 8:4057; Yelverton *et al.* (1981) *Nucl. Acids Res.* 9:731; U.S. Patent 4,738,921; EPO Publ. Nos. 036 776 and 121 775). The beta-lactamase (*bla*) promoter system (Weissmann (1981) "The cloning of interferon and other mistakes." In *Interferon 3* (ed. I. Gresser)), bacteriophage lambda PL (Shimatake *et al.* (1981) *Nature* 292:128) and T5 (U.S. Patent 4,689,406) promoter systems also provide useful promoter sequences.

In addition, synthetic promoters which do not occur in nature also function as bacterial promoters. For example, transcription activation sequences of one bacterial or bacteriophage promoter may be joined with the operon sequences of another bacterial or bacteriophage promoter, creating a synthetic hybrid promoter (U.S. Patent 4,551,433). For

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example, the *tac* promoter is a hybrid *trp-lac* promoter comprised of both *trp* promoter and *lac* operon sequences that is regulated by the *lac* repressor (Amann *et al.* (1983) *Gene* 25:167; de Boer *et al.* (1983) *Proc. Natl. Acad. Sci.* 80:21). Furthermore, a bacterial promoter can include naturally occurring promoters of non-bacterial origin that have the ability to bind bacterial RNA polymerase and initiate transcription. A naturally occurring promoter of non-bacterial origin can also be coupled with a compatible RNA polymerase to produce high levels of expression of some genes in prokaryotes. The bacteriophage T7 RNA polymerase/promoter system is an example of a coupled promoter system (Studier *et al.* (1986) *J. Mol. Biol.* 189:113; Tabor *et al.* (1985) *Proc Natl. Acad. Sci.* 82:1074). In addition, a hybrid promoter can also be comprised of a bacteriophage promoter and an *E. coli* operator region (EPO Publ. No. 267 851).

In addition to a functioning promoter sequence, an efficient ribosome binding site is also useful for the expression of foreign genes in prokaryotes. In *E. coli*, the ribosome binding site is called the Shine-Dalgarno (SD) sequence and includes an initiation codon (ATG) and a sequence 3-9 nucleotides in length located 3-11 nucleotides upstream of the initiation codon (Shine *et al.* (1975) *Nature* 254:34). The SD sequence is thought to promote binding of mRNA to the ribosome by the pairing of bases between the SD sequence and the 3' end of *E. coli* 16S rRNA (Steitz *et al.* (1979) "Genetic signals and nucleotide sequences in messenger RNA." In *Biological Regulation and Development: Gene Expression* (ed. R.F. Goldberger)). To express eukaryotic genes and prokaryotic genes with weak ribosome-binding site, it is often necessary to optimize the distance between the SD sequence and the ATG of the eukaryotic gene (Sambrook *et al.* (1989) "Expression of cloned genes in *Escherichia coli*." In *Molecular Cloning: A Laboratory Manual*).

A DNA molecule may be expressed intracellularly. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide or by either *in vivo* or *in vitro* incubation with a bacterial methionine N-terminal peptidase (EPO Publ. No. 219 237).

Fusion proteins provide an alternative to direct expression. Usually, a DNA sequence encoding the N-terminal portion of an endogenous bacterial protein, or other stable protein, is

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fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the bacteriophage lambda cell gene can be linked at the 5' terminus of a foreign gene and expressed in bacteria. The resulting fusion protein preferably retains a site for a processing enzyme (factor Xa) to cleave the bacteriophage protein from the foreign gene (Nagai *et al.* (1984) *Nature* 309:810). Fusion proteins can also be made with sequences from the *lacZ* (Jia *et al.* (1987) *Gene* 60:197), *trpE* (Allen *et al.* (1987) *J. Biotechnol.* 5:93; Makoff *et al.* (1989) *J. Gen. Microbiol.* 135:11), and *Chey* (EPO Publ. No. 324 647) genes. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (e.g. ubiquitin specific processing-protease) to cleave the ubiquitin from the foreign protein. Through this method, native foreign protein can be isolated (Miller *et al.* (1989) *Bio/Technology* 7:698).

Alternatively, foreign proteins can also be secreted from the cell by creating chimeric DNA molecules that encode a fusion protein comprised of a signal peptide sequence fragment that provides for secretion of the foreign protein in bacteria (U.S. Patent 4,336,336). The signal sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The protein is either secreted into the growth media (gram-positive bacteria) or into the periplasmic space, located between the inner and outer membrane of the cell (gram-negative bacteria). Preferably there are processing sites, which can be cleaved either *in vivo* or *in vitro* encoded between the signal peptide fragment and the foreign gene.

DNA encoding suitable signal sequences can be derived from genes for secreted bacterial proteins, such as the *E. coli* outer membrane protein gene (*ompA*) (Masui *et al.* (1983), in: *Experimental Manipulation of Gene Expression*; Ghayeb *et al.* (1984) *EMBO J.* 3:2437) and the *E. coli* alkaline phosphatase signal sequence (*phoA*) (Oka *et al.* (1985) *Proc. Natl. Acad. Sci.* 82:7212). As an additional example, the signal sequence of the alpha-amylase gene from various *Bacillus* strains can be used to secrete heterologous proteins from *B. subtilis* (Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EPO Publ. No. 244 042).

Usually, transcription termination sequences recognized by bacteria are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the



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coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Transcription termination sequences frequently include DNA sequences of about 50 nucleotides capable of forming stem loop structures that aid in terminating transcription. Examples include transcription termination sequences derived from genes with strong promoters, such as the *trp* gene in *E. coli* as well as other biosynthetic genes.

Usually, the above described components, comprising a promoter, signal sequence (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (e.g., plasmids) capable of stable maintenance in a host, such as bacteria. The replicon will have a replication system, thus allowing it to be maintained in a prokaryotic host either for expression or for cloning and amplification. In addition, a replicon may be either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably contain at least about 10, and more preferably at least about 20 plasmids. Either a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host.

Alternatively, the expression constructs can be integrated into the bacterial genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to the bacterial chromosome that allows the vector to integrate. Integrations appear to result from recombinations between homologous DNA in the vector and the bacterial chromosome. For example, integrating vectors constructed with DNA from various *Bacillus* strains integrate into the *Bacillus* chromosome (EPO Publ. No. 127 328). Integrating vectors may also be comprised of bacteriophage or transposon sequences.

Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of bacterial strains that have been transformed. Selectable markers can be expressed in the bacterial host and may include genes which render bacteria resistant to drugs such as ampicillin, chloramphenicol, erythromycin, kanamycin (neomycin), and tetracycline (Davies *et al.* (1978) *Annu. Rev. Microbiol.* 32:469). Selectable

markers may also include biosynthetic genes, such as those in the histidine, tryptophan, and leucine biosynthetic pathways.

Alternatively, some of the above described components can be put together in transformation vectors. Transformation vectors are usually comprised of a selectable marker that is either maintained in a replicon or developed into an integrating vector, as described above.

Expression and transformation vectors, either extra-chromosomal replicons or integrating vectors, have been developed for transformation into many bacteria. For example, expression vectors have been developed for, *inter alia*, the following bacteria: *Bacillus subtilis* (Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EPO Publ. Nos. 036 259 and 063 953; PCT Publ. No. WO 84/04541), *Escherichia coli* (Shimatake *et al.* (1981) *Nature* 292:128; Amann *et al.* (1985) *Gene* 40:183; Studier *et al.* (1986) *J. Mol. Biol.* 189:113; EPO Publ. Nos. 036 776, 136 829 and 136 907), *Streptococcus cremoris* (Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655); *Streptococcus lividans* (Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655), *Streptomyces lividans* (U.S. Patent 4,745,056).

Methods of introducing exogenous DNA into bacterial hosts are well-known in the art, and usually include either the transformation of bacteria treated with  $\text{CaCl}_2$  or other agents, such as divalent cations and DMSO. DNA can also be introduced into bacterial cells by electroporation. Transformation procedures usually vary with the bacterial species to be transformed. (See e.g., use of *Bacillus*: Masson *et al.* (1989) *FEMS Microbiol. Lett.* 60:273; Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EPO Publ. Nos. 036 259 and 063 953; PCT Publ. No. WO 84/04541; use of *Campylobacter*: Miller *et al.* (1988) *Proc. Natl. Acad. Sci.* 85:856; and Wang *et al.* (1990) *J. Bacteriol.* 172:949; use of *Escherichia coli*: Cohen *et al.* (1973) *Proc. Natl. Acad. Sci.* 69:2110; Dower *et al.* (1988) *Nucleic Acids Res.* 16:6127; Kushner (1978) "An improved method for transformation of *Escherichia coli* with ColE1-derived plasmids. In *Genetic Engineering: Proceedings of the International Symposium on Genetic Engineering* (eds. H.W. Boyer and S. Nicosia); Mandel *et al.* (1970) *J. Mol. Biol.* 53:159; Taketo (1988) *Biochim. Biophys. Acta* 949:318; use of *Lactobacillus*: Chassy *et al.* (1987) *FEMS Microbiol. Lett.* 44:173; use of *Pseudomonas*: Fiedler *et al.* (1988) *Anal. Biochem.* 170:38; use of *Staphylococcus*: Augustin *et al.* (1990) *FEMS Microbiol. Lett.* 66:203; use of *Streptococcus*: Barany *et al.* (1980) *J. Bacteriol.* 144:698;

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Harlander (1987) "Transformation of *Streptococcus lactis* by electroporation, in: *Streptococcal Genetics* (ed. J. Ferretti and R. Curtiss III); Perry *et al.* (1981) *Infect. Immun.* 32:1295; Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655; Somkuti *et al.* (1987) *Proc. 4th Evr. Cong. Biotechnology* 1:412.

v. Yeast Expression

Yeast expression systems are also known to one of ordinary skill in the art. A yeast promoter is any DNA sequence capable of binding yeast RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g. structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site (the "TATA Box") and a transcription initiation site. A yeast promoter may also have a second domain called an upstream activator sequence (UAS), which, if present, is usually distal to the structural gene. The UAS permits regulated (inducible) expression. Constitutive expression occurs in the absence of a UAS. Regulated expression may be either positive or negative, thereby either enhancing or reducing transcription.

Yeast is a fermenting organism with an active metabolic pathway, therefore sequences encoding enzymes in the metabolic pathway provide particularly useful promoter sequences. Examples include alcohol dehydrogenase (ADH) (EPO Publ. No. 284 044), enolase, glucokinase, glucose-6-phosphate isomerase, glyceraldehyde-3-phosphate-dehydrogenase (GAP or GAPDH), hexokinase, phosphofructokinase, 3-phosphoglycerate mutase, and pyruvate kinase (PyK) (EPO Publ. No. 329 203). The yeast *PHO5* gene, encoding acid phosphatase, also provides useful promoter sequences (Myanohara *et al.* (1983) *Proc. Natl. Acad. Sci. USA* 80:1).

In addition, synthetic promoters which do not occur in nature also function as yeast promoters. For example, UAS sequences of one yeast promoter may be joined with the transcription activation region of another yeast promoter, creating a synthetic hybrid promoter. Examples of such hybrid promoters include the ADH regulatory sequence linked to the GAP transcription activation region (U.S. Patent Nos. 4,876,197 and 4,880,734). Other examples of hybrid promoters include promoters which consist of the regulatory sequences of

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either the *ADH2*, *GAL4*, *GAL10*, OR *PHO5* genes, combined with the transcriptional activation region of a glycolytic enzyme gene such as GAP or PyK (EPO Publ. No. 164 556). Furthermore, a yeast promoter can include naturally occurring promoters of non-yeast origin that have the ability to bind yeast RNA polymerase and initiate transcription. Examples of such promoters include, *inter alia*, (Cohen *et al.* (1980) *Proc. Natl. Acad. Sci. USA* 77:1078; Henikoff *et al.* (1981) *Nature* 283:835; Hollenberg *et al.* (1981) *Curr. Topics Microbiol. Immunol.* 96:119; Hollenberg *et al.* (1979) "The Expression of Bacterial Antibiotic Resistance Genes in the Yeast *Saccharomyces cerevisiae*," in: *Plasmids of Medical, Environmental and Commercial Importance* (eds. K.N. Timmis and A. Puhler); Mercerau-Puigalon *et al.* (1980) *Gene* 11:163; Panthier *et al.* (1980) *Curr. Genet.* 2:109;).

A DNA molecule may be expressed intracellularly in yeast. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide.

Fusion proteins provide an alternative for yeast expression systems, as well as in mammalian, plant, baculovirus, and bacterial expression systems. Usually, a DNA sequence encoding the N-terminal portion of an endogenous yeast protein, or other stable protein, is fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the yeast or human superoxide dismutase (SOD) gene, can be linked at the 5' terminus of a foreign gene and expressed in yeast. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. See e.g., EPO Publ. No. 196056. Another example is a ubiquitin fusion-protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (e.g. ubiquitin-specific processing protease) to cleave the ubiquitin from the foreign protein. Through this method, therefore, native foreign protein can be isolated (e.g., WO88/024066).

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provide for secretion in yeast of the foreign protein. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can

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be cleaved either *in vivo* or *in vitro*. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell.

DNA encoding suitable signal sequences can be derived from genes for secreted yeast proteins, such as the yeast invertase gene (EPO Publ. No. 012 873; JPO Publ. No. 62:096,086) and the A-factor gene (U.S. Patent 4,588,684). Alternatively, leaders of non-yeast origin, such as an interferon leader, exist that also provide for secretion in yeast (EPO Publ. No. 060 057).

A preferred class of secretion leaders are those that employ a fragment of the yeast alpha-factor gene, which contains both a "pre" signal sequence, and a "pro" region. The types of alpha-factor fragments that can be employed include the full-length pre-pro alpha factor leader (about 83 amino acid residues) as well as truncated alpha-factor leaders (usually about 25 to about 50 amino acid residues) (U.S. Patent Nos. 4,546,083 and 4,870,008; EPO Publ. No. 324 274). Additional leaders employing an alpha-factor leader fragment that provides for secretion include hybrid alpha-factor leaders made with a presequence of a first yeast, but a pro-region from a second yeast alpha factor. (See e.g., PCT Publ. No. WO 89/02463.)

Usually, transcription termination sequences recognized by yeast are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminator sequence and other yeast-recognized termination sequences, such as those coding for glycolytic enzymes.

Usually, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (e.g., plasmids) capable of stable maintenance in a host, such as yeast or bacteria. The replicon may have two replication systems, thus allowing it to be maintained, for example, in yeast for expression and in a prokaryotic host for cloning and amplification. Examples of such yeast-bacteria shuttle vectors include YEp24 (Botstein *et al.* (1979) *Gene* 8:17-24), pCI/1 (Brake *et al.* (1984) *Proc. Natl. Acad. Sci USA* 81:4642-4646), and YRp17 (Stinchcomb *et al.* (1982) *J. Mol. Biol.* 158:157). In addition, a replicon may be

either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably have at least about 10, and more preferably at least about 20. Either a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host. See e.g., Brake *et al.*, *supra*.

Alternatively, the expression constructs can be integrated into the yeast genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to a yeast chromosome that allows the vector to integrate, and preferably contain two homologous sequences flanking the expression construct. Integrations appear to result from recombinations between homologous DNA in the vector and the yeast chromosome (Orr-Weaver *et al.* (1983) *Methods in Enzymol.* 101:228-245). An integrating vector may be directed to a specific locus in yeast by selecting the appropriate homologous sequence for inclusion in the vector. See Orr-Weaver *et al.*, *supra*. One or more expression construct may integrate, possibly affecting levels of recombinant protein produced (Rine *et al.* (1983) *Proc. Natl. Acad. Sci. USA* 80:6750). The chromosomal sequences included in the vector can occur either as a single segment in the vector, which results in the integration of the entire vector, or two segments homologous to adjacent segments in the chromosome and flanking the expression construct in the vector, which can result in the stable integration of only the expression construct.

Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of yeast strains that have been transformed. Selectable markers may include biosynthetic genes that can be expressed in the yeast host, such as *ADE2*, *HIS4*, *LEU2*, *TRP1*, and *ALG7*, and the G418 resistance gene, which confer resistance in yeast cells to tunicamycin and G418, respectively. In addition, a suitable selectable marker may also provide yeast with the ability to grow in the presence of toxic compounds, such as metal. For example, the presence of *CUP1* allows yeast to grow in the presence of copper ions (Butt *et al.* (1987) *Microbiol. Rev.* 51:351).

Alternatively, some of the above described components can be put together into transformation vectors. Transformation vectors are usually comprised of a selectable marker

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that is either maintained in a replicon or developed into an integrating vector, as described above.

Expression and transformation vectors, either extrachromosomal replicons or integrating vectors, have been developed for transformation into many yeasts. For example, expression vectors and methods of introducing exogenous DNA into yeast hosts have been developed for, *inter alia*, the following yeasts: *Candida albicans* (Kurtz, *et al.* (1986) *Mol. Cell. Biol.* 6:142); *Candida maltosa* (Kunze, *et al.* (1985) *J. Basic Microbiol.* 25:141); *Hansenula polymorpha* (Gleeson, *et al.* (1986) *J. Gen. Microbiol.* 132:3459; Roggenkamp *et al.* (1986) *Mol. Gen. Genet.* 202:302); *Kluyveromyces fragilis* (Das, *et al.* (1984) *J. Bacteriol.* 158:1165); *Kluyveromyces lactis* (De Louvencourt *et al.* (1983) *J. Bacteriol.* 154:737; Van den Berg *et al.* (1990) *Bio/Technology* 8:135); *Pichia guilliermondii* (Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141); *Pichia pastoris* (Cregg, *et al.* (1985) *Mol. Cell. Biol.* 5:3376; U.S. Patent Nos. 4,837,148 and 4,929,555); *Saccharomyces cerevisiae* (Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA* 75:1929; Ito *et al.* (1983) *J. Bacteriol.* 153:163); *Schizosaccharomyces pombe* (Beach and Nurse (1981) *Nature* 300:706); and *Yarrowia lipolytica* (Davidow, *et al.* (1985) *Curr. Genet.* 10:380471 Gaillardin, *et al.* (1985) *Curr. Genet.* 10:49).

Methods of introducing exogenous DNA into yeast hosts are well-known in the art, and usually include either the transformation of spheroplasts or of intact yeast cells treated with alkali cations. Transformation procedures usually vary with the yeast species to be transformed. See e.g., [Kurtz *et al.* (1986) *Mol. Cell. Biol.* 6:142; Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141; *Candida*]; [Gleeson *et al.* (1986) *J. Gen. Microbiol.* 132:3459; Roggenkamp *et al.* (1986) *Mol. Gen. Genet.* 202:302; *Hansenula*]; [Das *et al.* (1984) *J. Bacteriol.* 158:1165; De Louvencourt *et al.* (1983) *J. Bacteriol.* 154:1165; Van den Berg *et al.* (1990) *Bio/Technology* 8:135; *Kluyveromyces*]; [Cregg *et al.* (1985) *Mol. Cell. Biol.* 5:3376; Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141; U.S. Patent Nos. 4,837,148 and 4,929,555; *Pichia*]; [Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA* 75:1929; Ito *et al.* (1983) *J. Bacteriol.* 153:163 *Saccharomyces*]; [Beach and Nurse (1981) *Nature* 300:706; *Schizosaccharomyces*]; [Davidow *et al.* (1985) *Curr. Genet.* 10:39; Gaillardin *et al.* (1985) *Curr. Genet.* 10:49; *Yarrowia*].

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### Definitions

A composition containing X is "substantially free of" Y when at least 85% by weight of the total X+Y in the composition is X. Preferably, X comprises at least about 90% by weight of the total of X+Y in the composition, more preferably at least about 95% or even 99% by weight.

The term "heterologous" refers to two biological components that are not found together in nature. The components may be host cells, genes, or regulatory regions, such as promoters. Although the heterologous components are not found together in nature, they can function together, as when a promoter heterologous to a gene is operably linked to the gene. Another example is where a Neisserial sequence is heterologous to a mouse host cell.

An "origin of replication" is a polynucleotide sequence that initiates and regulates replication of polynucleotides, such as an expression vector. The origin of replication behaves as an autonomous unit of polynucleotide replication within a cell, capable of replication under its own control. An origin of replication may be needed for a vector to replicate in a particular host cell. With certain origins of replication, an expression vector can be reproduced at a high copy number in the presence of the appropriate proteins within the cell. Examples of origins are the autonomously replicating sequences, which are effective in yeast; and the viral T-antigen, effective in COS-7 cells.

A "mutant" sequence is defined as a DNA, RNA or amino acid sequence differing from but having homology with the native or disclosed sequence. Depending on the particular sequence, the degree of homology between the native or disclosed sequence and the mutant sequence is preferably greater than 50% (e.g., 60%, 70%, 80%, 90%, 95%, 99% or more) which is calculated as described above. As used herein, an "allelic variant" of a nucleic acid molecule, or region, for which nucleic acid sequence is provided herein is a nucleic acid molecule, or region, that occurs at essentially the same locus in the genome of another or second isolate, and that, due to natural variation caused by, for example, mutation or recombination, has a similar but not identical nucleic acid sequence. A coding region allelic variant typically encodes a protein having similar activity to that of the protein encoded by the gene to which it is being compared. An allelic variant can also comprise an alteration in the 5' or 3' untranslated regions of the gene, such as in regulatory control regions. (see, for example, U.S. Patent 5,753,235).



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## Antibodies

As used herein, the term "antibody" refers to a polypeptide or group of polypeptides composed of at least one antibody combining site. An "antibody combining site" is the three-dimensional binding space with an internal surface shape and charge distribution complementary to the features of an epitope of an antigen, which allows a binding of the antibody with the antigen. "Antibody" includes, for example, vertebrate antibodies, hybrid antibodies, chimeric antibodies, humanized antibodies, altered antibodies, univalent antibodies, Fab proteins, and single domain antibodies.

Antibodies against the proteins of the invention are useful for affinity chromatography, immunoassays, and distinguishing/identifying *Neisseria* MenB proteins. Antibodies elicited against the proteins of the present invention bind to antigenic polypeptides or proteins or protein fragments that are present and specifically associated with strains of *Neisseria meningitidis* MenB. In some instances, these antigens may be associated with specific strains, such as those antigens specific for the MenB strains. The antibodies of the invention may be immobilized to a matrix and utilized in an immunoassay or on an affinity chromatography column, to enable the detection and/or separation of polypeptides, proteins or protein fragments or cells comprising such polypeptides, proteins or protein fragments. Alternatively, such polypeptides, proteins or protein fragments may be immobilized so as to detect antibodies bindably specific thereto.

Antibodies to the proteins of the invention, both polyclonal and monoclonal, may be prepared by conventional methods. In general, the protein is first used to immunize a suitable animal, preferably a mouse, rat, rabbit or goat. Rabbits and goats are preferred for the preparation of polyclonal sera due to the volume of serum obtainable, and the availability of labeled anti-rabbit and anti-goat antibodies. Immunization is generally performed by mixing or emulsifying the protein in saline, preferably in an adjuvant such as Freund's complete adjuvant, and injecting the mixture or emulsion parenterally (generally subcutaneously or intramuscularly). A dose of 50-200 µg/injection is typically sufficient. Immunization is generally boosted 2-6 weeks later with one or more injections of the protein in saline, preferably using Freund's incomplete adjuvant. One may alternatively generate antibodies by in vitro immunization using methods known in the art, which for the purposes of this

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invention is considered equivalent to *in vivo* immunization. Polyclonal antisera is obtained by bleeding the immunized animal into a glass or plastic container, incubating the blood at 25°C for one hour, followed by incubating at 4°C for 2-18 hours. The serum is recovered by centrifugation (e.g., 1,000g for 10 minutes). About 20-50 ml per bleed may be obtained from rabbits.

Monoclonal antibodies are prepared using the standard method of Kohler & Milstein (*Nature* (1975) 256:495-96), or a modification thereof. Typically, a mouse or rat is immunized as described above. However, rather than bleeding the animal to extract serum, the spleen (and optionally several large lymph nodes) is removed and dissociated into single cells. If desired, the spleen cells may be screened (after removal of nonspecifically adherent cells) by applying a cell suspension to a plate or well coated with the protein antigen. B-cells that express membrane-bound immunoglobulin specific for the antigen bind to the plate, and are not rinsed away with the rest of the suspension. Resulting B-cells, or all dissociated spleen cells, are then induced to fuse with myeloma cells to form hybridomas, and are cultured in a selective medium (e.g., hypoxanthine, aminopterin, thymidine medium, "HAT"). The resulting hybridomas are plated by limiting dilution, and are assayed for the production of antibodies which bind specifically to the immunizing antigen (and which do not bind to unrelated antigens). The selected MAb-secreting hybridomas are then cultured either *in vitro* (e.g., in tissue culture bottles or hollow fiber reactors), or *in vivo* (as ascites in mice).

If desired, the antibodies (whether polyclonal or monoclonal) may be labeled using conventional techniques. Suitable labels include fluorophores, chromophores, radioactive atoms (particularly  $^{32}\text{P}$  and  $^{125}\text{I}$ ), electron-dense reagents, enzymes, and ligands having specific binding partners. Enzymes are typically detected by their activity. For example, horseradish peroxidase is usually detected by its ability to convert 3,3',5,5'-tetramethylbenzidine (TMB) to a blue pigment, quantifiable with a spectrophotometer. "Specific binding partner" refers to a protein capable of binding a ligand molecule with high specificity, as for example in the case of an antigen and a monoclonal antibody specific therefor. Other specific binding partners include biotin and avidin or streptavidin, IgG and protein A, and the numerous receptor-ligand couples known in the art. It should be understood that the above description is not meant to categorize the various

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labels into distinct classes, as the same label may serve in several different modes. For example,  $^{125}\text{I}$  may serve as a radioactive label or as an electron-dense reagent. HRP may serve as enzyme or as antigen for a MAb. Further, one may combine various labels for desired effect. For example, MAbs and avidin also require labels in the practice of this invention: thus, one might label a MAb with biotin, and detect its presence with avidin labeled with  $^{125}\text{I}$ , or with an anti-biotin MAb labeled with HRP. Other permutations and possibilities will be readily apparent to those of ordinary skill in the art, and are considered as equivalents within the scope of the instant invention.

Antigens, immunogens, polypeptides, proteins or protein fragments of the present invention elicit formation of specific binding partner antibodies. These antigens, immunogens, polypeptides, proteins or protein fragments of the present invention comprise immunogenic compositions of the present invention. Such immunogenic compositions may further comprise or include adjuvants, carriers, or other compositions that promote or enhance or stabilize the antigens, polypeptides, proteins or protein fragments of the present invention. Such adjuvants and carriers will be readily apparent to those of ordinary skill in the art.

#### Pharmaceutical Compositions

Pharmaceutical compositions can include either polypeptides, antibodies, or nucleic acid of the invention. The pharmaceutical compositions will comprise a therapeutically effective amount of either polypeptides, antibodies, or polynucleotides of the claimed invention.

The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic or preventative effect. The effect can be detected by, for example, chemical markers or antigen levels. Therapeutic effects also include reduction in physical symptoms, such as decreased body temperature, when given to a patient that is febrile. The precise effective amount for a subject will depend upon the subject's size and health, the nature and extent of the condition, and the therapeutics or combination of therapeutics selected for administration. Thus, it is not useful to specify an exact effective amount in

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advance. However, the effective amount for a given situation can be determined by routine experimentation and is within the judgment of the clinician.

For purposes of the present invention, an effective dose will be from about 0.01 mg/kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

A pharmaceutical composition can also contain a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent, such as antibodies or a polypeptide, genes, and other therapeutic agents. The term refers to any pharmaceutical carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition, and which may be administered without undue toxicity. Suitable carriers may be large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Such carriers are well known to those of ordinary skill in the art.

Pharmaceutically acceptable salts can be used therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

Pharmaceutically acceptable carriers in therapeutic compositions may contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. Typically, the therapeutic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. Liposomes are included within the definition of a pharmaceutically acceptable carrier.

#### Delivery Methods

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals; in particular, human subjects can be treated.

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Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal and transcutaneous applications, needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule.

### Vaccines

Vaccines according to the invention may either be prophylactic (i.e., to prevent infection) or therapeutic (i.e., to treat disease after infection).

Such vaccines comprise immunizing antigen(s) or immunogen(s), immunogenic polypeptide, protein(s) or protein fragments, or nucleic acids (e.g., ribonucleic acid or deoxyribonucleic acid), usually in combination with "pharmaceutically acceptable carriers," which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, lipid aggregates (such as oil droplets or liposomes), and inactive virus particles. Such carriers are well known to those of ordinary skill in the art. Additionally, these carriers may function as immunostimulating agents ("adjuvants"). Furthermore, the immunogen or antigen may be conjugated to a bacterial toxoid, such as a toxoid from diphtheria, tetanus, cholera, *H. pylori*, etc. pathogens.

Preferred adjuvants to enhance effectiveness of the composition include, but are not limited to: (1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc; (2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59 (PCT Publ. No. WO 90/14837), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE (see below), although not required) formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA), (b) SAF, containing 10% Squalene, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP (see below) either microfluidized into a submicron emulsion or vortexed to generate a

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larger particle size emulsion, and (c) Ribi<sup>TM</sup> adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox<sup>TM</sup>); (3) saponin adjuvants, such as Stimulon<sup>TM</sup> (Cambridge Bioscience, Worcester, MA) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes); (4) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (5) cytokines, such as interleukins (e.g., IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, *etc.*), interferons (e.g., gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), *etc.*; (6) detoxified mutants of a bacterial ADP-ribosylating toxin such as a cholera toxin (CT), a pertussis toxin (PT), or an *E. coli* heat-labile toxin (LT), particularly LT-K63, LT-R72, CT-S109, PT-K9/G129; see, e.g., WO 93/13302 and WO 92/19265; and (7) other substances that act as immunostimulating agents to enhance the effectiveness of the composition. Alum and MF59 are preferred.

As mentioned above, muramyl peptides include, but are not limited to, N-acetylmuramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), *etc.*

The vaccine compositions comprising immunogenic compositions (e.g., which may include the antigen, pharmaceutically acceptable carrier, and adjuvant) typically will contain diluents, such as water, saline, glycerol, ethanol, *etc.* Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. Alternatively, vaccine compositions comprising immunogenic compositions may comprise an antigen, polypeptide, protein, protein fragment or nucleic acid in a pharmaceutically acceptable carrier.

More specifically, vaccines comprising immunogenic compositions comprise an immunologically effective amount of the immunogenic polypeptides, as well as any other of the above-mentioned components, as needed. By "immunologically effective amount", it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, the taxonomic group of

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individual to be treated (e.g., nonhuman primate, primate, *etc.*), the capacity of the individual's immune system to synthesize antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

Typically, the vaccine compositions or immunogenic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation also may be emulsified or encapsulated in liposomes for enhanced adjuvant effect, as discussed above under pharmaceutically acceptable carriers.

The immunogenic compositions are conventionally administered parenterally, e.g., by injection, either subcutaneously or intramuscularly. Additional formulations suitable for other modes of administration include oral and pulmonary formulations, suppositories, and transdermal and transcutaneous applications. Dosage treatment may be a single dose schedule or a multiple dose schedule. The vaccine may be administered in conjunction with other immunoregulatory agents.

As an alternative to protein-based vaccines, DNA vaccination may be employed (e.g., Robinson & Torres (1997) *Seminars in Immunology* 9:271-283; Donnelly *et al.* (1997) *Annu Rev Immunol* 15:617-648).

#### Gene Delivery Vehicles

Gene therapy vehicles for delivery of constructs, including a coding sequence of a therapeutic of the invention, to be delivered to the mammal for expression in the mammal, can be administered either locally or systemically. These constructs can utilize viral or non-viral vector approaches in *in vivo* or *ex vivo* modality. Expression of such coding sequence can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence in vivo can be either constitutive or regulated.

The invention includes gene delivery vehicles capable of expressing the contemplated nucleic acid sequences. The gene delivery vehicle is preferably a viral vector and, more preferably, a retroviral, adenoviral, adeno-associated viral (AAV), herpes viral, or alphavirus vector. The viral vector can also be an astrovirus, coronavirus, orthomyxovirus, papovavirus,

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paramyxovirus, parvovirus, picornavirus, poxvirus, or togavirus viral vector. See generally, Jolly (1994) *Cancer Gene Therapy* 1:51-64; Kimura (1994) *Human Gene Therapy* 5:845-852; Connelly (1995) *Human Gene Therapy* 6:185-193; and Kaplitt (1994) *Nature Genetics* 6:148-153.

Retroviral vectors are well known in the art, including B, C and D type retroviruses, xenotropic retroviruses (for example, NZB-X1, NZB-X2 and NZB9-1 (see O'Neill (1985) *J. Virol.* 53:160) polytropic retroviruses e.g., MCF and MCF-MLV (see Kelly (1983) *J. Virol.* 45:291), spumaviruses and lentiviruses. See RNA Tumor Viruses, Second Edition, Cold Spring Harbor Laboratory, 1985.

Portions of the retroviral gene therapy vector may be derived from different retroviruses. For example, retrovector LTRs may be derived from a Murine Sarcoma Virus, a tRNA binding site from a Rous Sarcoma Virus, a packaging signal from a Murine Leukemia Virus, and an origin of second strand synthesis from an Avian Leukosis Virus.

These recombinant retroviral vectors may be used to generate transduction competent retroviral vector particles by introducing them into appropriate packaging cell lines (see US patent 5,591,624). Retrovirus vectors can be constructed for site-specific integration into host cell DNA by incorporation of a chimeric integrase enzyme into the retroviral particle (see WO96/37626). It is preferable that the recombinant viral vector is a replication defective recombinant virus.

Packaging cell lines suitable for use with the above-described retrovirus vectors are well known in the art, are readily prepared (see WO95/30763 and WO92/05266), and can be used to create producer cell lines (also termed vector cell lines or "VCLs") for the production of recombinant vector particles. Preferably, the packaging cell lines are made from human parent cells (e.g., HT1080 cells) or mink parent cell lines, which eliminates inactivation in human serum.

Preferred retroviruses for the construction of retroviral gene therapy vectors include Avian Leukosis Virus, Bovine Leukemia Virus, Murine Leukemia Virus, Mink-Cell Focus-Inducing Virus, Murine Sarcoma Virus, Reticuloendotheliosis Virus and Rous Sarcoma Virus. Particularly preferred Murine Leukemia Viruses include 4070A and 1504A (Hartley and Rowe (1976) *J Virol* 19:19-25), Abelson (ATCC No. VR-999), Friend (ATCC No. VR-245), Graffi, Gross (ATCC No.1 VR-590), Kirsten, Harvey Sarcoma Virus and



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Rauscher (ATCC No. VR-998) and Moloney Murine Leukemia Virus (ATCC No. VR-190). Such retroviruses may be obtained from depositories or collections such as the American Type Culture Collection ("ATCC") in Rockville, Maryland or isolated from known sources using commonly available techniques.

Exemplary known retroviral gene therapy vectors employable in this invention include those described in patent applications GB2200651, EP0415731, EP0345242, EP0334301, WO89/02468; WO89/05349, WO89/09271, WO90/02806, WO90/07936, WO94/03622, WO93/25698, WO93/25234, WO93/11230, WO93/10218, WO91/02805, WO91/02825, WO95/07994, US 5,219,740, US 4,405,712, US 4,861,719, US 4,980,289, US 4,777,127, US 5,591,624. See also Vile (1993) *Cancer Res* 53:3860-3864; Vile (1993) *Cancer Res* 53:962-967; Ram (1993) *Cancer Res* 53 (1993) 83-88; Takamiya (1992) *J Neurosci Res* 33:493-503; Baba (1993) *J Neurosurg* 79:729-735; Mann (1983) *Cell* 33:153; Cane (1984) *Proc Natl Acad Sci* 81:6349; and Miller (1990) *Human Gene Therapy* 1.

Human adenoviral gene therapy vectors are also known in the art and employable in this invention. See, for example, Berkner (1988) *Biotechniques* 6:616 and Rosenfeld (1991) *Science* 252:431, and WO93/07283, WO93/06223, and WO93/07282. Exemplary known adenoviral gene therapy vectors employable in this invention include those described in the above referenced documents and in WO94/12649, WO93/03769, WO93/19191, WO94/28938, WO95/11984, WO95/00655, WO95/27071, WO95/29993, WO95/34671, WO96/05320, WO94/08026, WO94/11506, WO93/06223, WO94/24299, WO95/14102, WO95/24297, WO95/02697, WO94/28152, WO94/24299, WO95/09241, WO95/25807, WO95/05835, WO94/18922 and WO95/09654. Alternatively, administration of DNA linked to killed adenovirus as described in Curiel (1992) *Hum. Gene Ther.* 3:147-154 may be employed. The gene delivery vehicles of the invention also include adenovirus associated virus (AAV) vectors. Leading and preferred examples of such vectors for use in this invention are the AAV-2 based vectors disclosed in Srivastava, WO93/09239. Most preferred AAV vectors comprise the two AAV inverted terminal repeats in which the native D-sequences are modified by substitution of nucleotides, such that at least 5 native nucleotides and up to 18 native nucleotides, preferably at least 10 native nucleotides up to 18 native nucleotides, most preferably 10 native nucleotides are retained and the remaining nucleotides of the D-sequence are deleted or replaced with non-native nucleotides. The native

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D-sequences of the AAV inverted terminal repeats are sequences of 20 consecutive nucleotides in each AAV inverted terminal repeat (i.e., there is one sequence at each end) which are not involved in HP formation. The non-native replacement nucleotide may be any nucleotide other than the nucleotide found in the native D-sequence in the same position. Other employable exemplary AAV vectors are pWP-19, pWN-1, both of which are disclosed in Nahreini (1993) *Gene* 124:257-262. Another example of such an AAV vector is psub201 (see Samulski (1987) *J. Virol.* 61:3096). Another exemplary AAV vector is the Double-D ITR vector. Construction of the Double-D ITR vector is disclosed in US Patent 5,478,745. Still other vectors are those disclosed in Carter US Patent 4,797,368 and Muzyczka US Patent 5,139,941, Chartejee US Patent 5,474,935, and Kotin WO94/288157. Yet a further example of an AAV vector employable in this invention is SSV9AFABTKneo, which contains the AFP enhancer and albumin promoter and directs expression predominantly in the liver. Its structure and construction are disclosed in Su (1996) *Human Gene Therapy* 7:463-470. Additional AAV gene therapy vectors are described in US 5,354,678, US 5,173,414, US 5,139,941, and US 5,252,479.

The gene therapy vectors comprising sequences of the invention also include herpes vectors. Leading and preferred examples are herpes simplex virus vectors containing a sequence encoding a thymidine kinase polypeptide such as those disclosed in US 5,288,641 and EP0176170 (Roizman). Additional exemplary herpes simplex virus vectors include HFEM/ICP6-LacZ disclosed in WO95/04139 (Wistar Institute), pHSVlac described in Geller (1988) *Science* 241:1667-1669 and in WO90/09441 and WO92/07945, HSV Us3::pgC-lacZ described in Fink (1992) *Human Gene Therapy* 3:11-19 and HSV 7134, 2 RH 105 and GAL4 described in EP 0453242 (Breakefield), and those deposited with the ATCC as accession numbers ATCC VR-977 and ATCC VR-260.

Also contemplated are alpha virus gene therapy vectors that can be employed in this invention. Preferred alpha virus vectors are Sindbis viruses vectors. Togaviruses, Semliki Forest virus (ATCC VR-67; ATCC VR-1247), Middleberg virus (ATCC VR-370), Ross River virus (ATCC VR-373; ATCC VR-1246), Venezuelan equine encephalitis virus (ATCC VR923; ATCC VR-1250; ATCC VR-1249; ATCC VR-532), and those described in US patents 5,091,309, 5,217,879, and WO92/10578. More particularly, those alpha virus vectors described in U.S. Serial No. 08/405,627, filed March 15, 1995, WO94/21792, WO92/10578,

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WO95/07994, US 5,091,309 and US 5,217,879 are employable. Such alpha viruses may be obtained from depositories or collections such as the ATCC in Rockville, Maryland or isolated from known sources using commonly available techniques. Preferably, alphavirus vectors with reduced cytotoxicity are used (see USSN 08/679640).

DNA vector systems such as eukaryotic layered expression systems are also useful for expressing the nucleic acids of the invention. See WO95/07994 for a detailed description of eukaryotic layered expression systems. Preferably, the eukaryotic layered expression systems of the invention are derived from alphavirus vectors and most preferably from Sindbis viral vectors.

Other viral vectors suitable for use in the present invention include those derived from poliovirus, for example ATCC VR-58 and those described in Evans, *Nature* 339 (1989) 385 and Sabin (1973) *J. Biol. Standardization* 1:115; rhinovirus, for example ATCC VR-1110 and those described in Arnold (1990) *J Cell Biochem* L401; pox viruses such as canary pox virus or vaccinia virus, for example ATCC VR-111 and ATCC VR-2010 and those described in Fisher-Hoch (1989) *Proc Natl Acad Sci* 86:317; Flexner (1989) *Ann NY Acad Sci* 569:86, Flexner (1990) *Vaccine* 8:17; in US 4,603,112 and US 4,769,330 and WO89/01973; SV40 virus, for example ATCC VR-305 and those described in Mulligan (1979) *Nature* 277:108 and Madzak (1992) *J Gen Virol* 73:1533; influenza virus, for example ATCC VR-797 and recombinant influenza viruses made employing reverse genetics techniques as described in US 5,166,057 and in Enami (1990) *Proc Natl Acad Sci* 87:3802-3805; Enami & Palese (1991) *J Virol* 65:2711-2713 and Luytjes (1989) *Cell* 59:110, (see also McMichael (1983) *NEJ Med* 309:13, and Yap (1978) *Nature* 273:238 and *Nature* (1979) 277:108); human immunodeficiency virus as described in EP-0386882 and in Buchschacher (1992) *J. Virol.* 66:2731; measles virus, for example ATCC VR-67 and VR-1247 and those described in EP-0440219; Aura virus, for example ATCC VR-368; Bebaru virus, for example ATCC VR-600 and ATCC VR-1240; Cabassou virus, for example ATCC VR-922; Chikungunya virus, for example ATCC VR-64 and ATCC VR-1241; Fort Morgan Virus, for example ATCC VR-924; Getah virus, for example ATCC VR-369 and ATCC VR-1243; Kyzylagach virus, for example ATCC VR-927; Mayaro virus, for example ATCC VR-66; Mucambo virus, for example ATCC VR-580 and ATCC VR-1244; Ndumu virus, for example ATCC VR-371; Pixuna virus, for example ATCC VR-372 and ATCC VR-1245; Tonate virus, for example

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ATCC VR-925; Trinit virus, for example ATCC VR-469; Una virus, for example ATCC VR-374; Whataroa virus, for example ATCC VR-926; Y-62-33 virus, for example ATCC VR-375; O'Nyong virus, Eastern encephalitis virus, for example ATCC VR-65 and ATCC VR-1242; Western encephalitis virus, for example ATCC VR-70, ATCC VR-1251, ATCC VR-622 and ATCC VR-1252; and coronavirus, for example ATCC VR-740 and those described in Hamre (1966) *Proc Soc Exp Biol Med* 121:190.

Delivery of the compositions of this invention into cells is not limited to the above mentioned viral vectors. Other delivery methods and media may be employed such as, for example, nucleic acid expression vectors, polycationic condensed DNA linked or unlinked to killed adenovirus alone, for example see US Serial No. 08/366,787, filed December 30, 1994 and Curiel (1992) *Hum Gene Ther* 3:147-154 ligand linked DNA, for example see Wu (1989) *J Biol Chem* 264:16985-16987, eucaryotic cell delivery vehicles cells, for example see US Serial No. 08/240,030, filed May 9, 1994, and US Serial No. 08/404,796, deposition of photopolymerized hydrogel materials, hand-held gene transfer particle gun, as described in US Patent 5,149,655, ionizing radiation as described in US 5,206,152 and in WO92/11033, nucleic charge neutralization or fusion with cell membranes. Additional approaches are described in Philip (1994) *Mol Cell Biol* 14:2411-2418 and in Woffendin (1994) *Proc Natl Acad Sci* 91:1581-1585.

Particle mediated gene transfer may be employed, for example see US Serial No. 60/023,867. Briefly, the sequence can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, as described in Wu & Wu (1987) *J. Biol. Chem.* 262:4429-4432, insulin as described in Hucked (1990) *Biochem Pharmacol* 40:253-263, galactose as described in Plank (1992) *Bioconjugate Chem* 3:533-539, lactose or transferrin.

Naked DNA may also be employed to transform a host cell. Exemplary naked DNA introduction methods are described in WO 90/11092 and US 5,580,859. Uptake efficiency may be improved using biodegradable latex beads. DNA coated latex beads are efficiently transported into cells after endocytosis initiation by the beads. The method may be improved

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further by treatment of the beads to increase hydrophobicity and thereby facilitate disruption of the endosome and release of the DNA into the cytoplasm.

Liposomes that can act as gene delivery vehicles are described in U.S. 5,422,120, WO95/13796, WO94/23697, WO91/14445 and EP-524,968. As described in USSN. 60/023,867, on non-viral delivery, the nucleic acid sequences encoding a polypeptide can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then be incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, insulin, galactose, lactose, or transferrin. Other delivery systems include the use of liposomes to encapsulate DNA comprising the gene under the control of a variety of tissue-specific or ubiquitously-active promoters. Further non-viral delivery suitable for use includes mechanical delivery systems such as the approach described in Woffendin *et al* (1994) *Proc. Natl. Acad. Sci. USA* 91(24):11581-11585. Moreover, the coding sequence and the product of expression of such can be delivered through deposition of photopolymerized hydrogel materials. Other conventional methods for gene delivery that can be used for delivery of the coding sequence include, for example, use of hand-held gene transfer particle gun, as described in U.S. 5,149,655; use of ionizing radiation for activating transferred gene, as described in U.S. 5,206,152 and WO92/11033

Exemplary liposome and polycationic gene delivery vehicles are those described in US 5,422,120 and 4,762,915; in WO 95/13796; WO94/23697; and WO91/14445; in EP-0524968; and in Stryer, *Biochemistry*, pages 236-240 (1975) W.H. Freeman, San Francisco; Szoka (1980) *Biochem Biophys Acta* 600:1; Bayer (1979) *Biochem Biophys Acta* 550:464; Rivnay (1987) *Meth Enzymol* 149:119; Wang (1987) *Proc Natl Acad Sci* 84:7851; Plant (1989) *Anal Biochem* 176:420.

A polynucleotide composition can comprise a therapeutically effective amount of a gene therapy vehicle, as the term is defined above. For purposes of the present invention, an effective dose will be from about 0.01 mg/ kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

### Delivery Methods

Once formulated, the polynucleotide compositions of the invention can be administered (1) directly to the subject; (2) delivered *ex vivo*, to cells derived from the subject; or (3) *in vitro* for expression of recombinant proteins. The subjects to be treated can be mammals or birds. Also, human subjects can be treated.

Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, transdermally or transcutaneously, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a tumor or lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal applications, needles, and gene guns or hypodermic sprays. Dosage treatment may be a single dose schedule or a multiple dose schedule. See WO98/20734.

Methods for the *ex vivo* delivery and reimplantation of transformed cells into a subject are known in the art and described in e.g., WO93/14778. Examples of cells useful in *ex vivo* applications include, for example, stem cells, particularly hematopoietic, lymph cells, macrophages, dendritic cells, or tumor cells.

Generally, delivery of nucleic acids for both *ex vivo* and *in vitro* applications can be accomplished by the following procedures, for example, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei, all well known in the art.

### Polynucleotide and Polypeptide pharmaceutical compositions

In addition to the pharmaceutically acceptable carriers and salts described above, the following additional agents can be used with polynucleotide and/or polypeptide compositions.

#### A. Polypeptides

One example are polypeptides which include, without limitation: asialoorosomucoid (ASOR); transferrin; asialoglycoproteins; antibodies; antibody fragments; ferritin; interleukins; interferons, granulocyte, macrophage colony stimulating factor (GM-CSF),

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granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), stem cell factor and erythropoietin. Viral antigens, such as envelope proteins, can also be used. Also, proteins from other invasive organisms, such as the 17 amino acid peptide from the circumsporozoite protein of *Plasmodium falciparum* known as RII.

B. Hormones, Vitamins, Etc.

Other groups that can be included in a pharmaceutical composition include, for example: hormones, steroids, androgens, estrogens, thyroid hormone, or vitamins, folic acid.

C. Polyalkylenes, Polysaccharides, etc.

Also, polyalkylene glycol can be included in a pharmaceutical compositions with the desired polynucleotides and/or polypeptides. In a preferred embodiment, the polyalkylene glycol is polyethylene glycol. In addition, mono-, di-, or polysaccharides can be included. In a preferred embodiment of this aspect, the polysaccharide is dextran or DEAE-dextran. Also, chitosan and poly(lactide-co-glycolide) may be included in a pharmaceutical composition.

D. Lipids, and Liposomes

The desired polynucleotide or polypeptide can also be encapsulated in lipids or packaged in liposomes prior to delivery to the subject or to cells derived therefrom.

Lipid encapsulation is generally accomplished using liposomes which are able to stably bind or entrap and retain nucleic acid or polypeptide. The ratio of condensed polynucleotide to lipid preparation can vary but will generally be around 1:1 (mg DNA:micromoles lipid), or more of lipid. For a review of the use of liposomes as carriers for delivery of nucleic acids, see, Hug and Sleight (1991) *Biochim. Biophys. Acta.* 1097:1-17; Straubinger (1983) *Meth. Enzymol.* 101:512-527.

Liposomal preparations for use in the present invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner (1987) *Proc. Natl. Acad. Sci. USA* 84:7413-7416); mRNA (Malone (1989) *Proc. Natl. Acad. Sci. USA* 86:6077-6081); and purified transcription factors (Debs (1990) *J. Biol. Chem.* 265:10189-10192), in functional form.

Cationic liposomes are readily available. For example, N(1-2,3-dioleoyloxy)propyl)-N,N,N-triethylammonium (DOTMA) liposomes are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, NY. (See, also, Felgner *supra*). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer). Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g., Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; WO90/11092 for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, AL), or can be easily prepared using readily available materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

The liposomes can comprise multilammellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs). The various liposome-nucleic acid complexes are prepared using methods known in the art. See e.g., Straubinger (1983) *Meth. Immunol.* 101:512-527; Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; Papahadjopoulos (1975) *Biochim. Biophys. Acta* 394:483; Wilson (1979) *Cell* 17:77; Deamer & Bangham (1976) *Biochim. Biophys. Acta* 443:629; Ostro (1977) *Biochem. Biophys. Res. Commun.* 76:836; Fraley (1979) *Proc. Natl. Acad. Sci. USA* 76:3348; Enoch & Strittmatter (1979) *Proc. Natl. Acad. Sci. USA* 76:145; Fraley (1980) *J. Biol. Chem.* (1980) 255:10431; Szoka & Papahadjopoulos (1978) *Proc. Natl. Acad. Sci. USA* 75:145; and Schaefer-Ridder (1982) *Science* 215:166.

#### E. Lipoproteins

In addition, lipoproteins can be included with the polynucleotide or polypeptide to be delivered. Examples of lipoproteins to be utilized include: chylomicrons, HDL, IDL, LDL, and VLDL. Mutants, fragments, or fusions of these proteins can also be used. Also, modifications of naturally occurring lipoproteins can be used, such as acetylated LDL. These



lipoproteins can target the delivery of polynucleotides to cells expressing lipoprotein receptors. Preferably, if lipoproteins are including with the polynucleotide to be delivered, no other targeting ligand is included in the composition.

Naturally occurring lipoproteins comprise a lipid and a protein portion. The protein portion are known as apoproteins. At the present, apoproteins A, B, C, D, and E have been isolated and identified. At least two of these contain several proteins, designated by Roman numerals, AI, AII, AIV; CI, CII, CIII.

A lipoprotein can comprise more than one apoprotein. For example, naturally occurring chylomicrons comprises of A, B, C, and E; over time these lipoproteins lose A and acquire C and E apoproteins. VLDL comprises A, B, C, and E apoproteins, LDL comprises apoprotein B; and HDL comprises apoproteins A, C, and E.

The amino acid sequences of these apoproteins are known and are described in, for example, Breslow (1985) *Annu Rev. Biochem* 54:699; Law (1986) *Adv. Exp Med. Biol.* 151:162; Chen (1986) *J Biol Chem* 261:12918; Kane (1980) *Proc Natl Acad Sci USA* 77:2465; and Utermann (1984) *Hum Genet* 65:232.

Lipoproteins contain a variety of lipids including, triglycerides, cholesterol (free and esters), and phospholipids. The composition of the lipids varies in naturally occurring lipoproteins. For example, chylomicrons comprise mainly triglycerides. A more detailed description of the lipid content of naturally occurring lipoproteins can be found, for example, in *Meth. Enzymol.* 128 (1986). The composition of the lipids are chosen to aid in conformation of the apoprotein for receptor binding activity. The composition of lipids can also be chosen to facilitate hydrophobic interaction and association with the polynucleotide binding molecule.

Naturally occurring lipoproteins can be isolated from serum by ultracentrifugation, for instance. Such methods are described in *Meth. Enzymol. (supra)*; Pitas (1980) *J. Biochem.* 255:5454-5460 and Mahey (1979) *J Clin. Invest* 64:743-750.

Lipoproteins can also be produced by *in vitro* or recombinant methods by expression of the apoprotein genes in a desired host cell. See, for example, Atkinson (1986) *Annu Rev Biophys Chem* 15:403 and Radding (1958) *Biochim Biophys Acta* 30: 443.

Lipoproteins can also be purchased from commercial suppliers, such as Biomedical Technologies, Inc., Stoughton, Massachusetts, USA.

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Further description of lipoproteins can be found in Zuckermann et al., PCT. Appln. No. US97/14465.

#### F. Polycationic Agents

Polycationic agents can be included, with or without lipoprotein, in a composition with the desired polynucleotide and/or polypeptide to be delivered.

Polycationic agents, typically, exhibit a net positive charge at physiological relevant pH and are capable of neutralizing the electrical charge of nucleic acids to facilitate delivery to a desired location. These agents have both in vitro, ex vivo, and in vivo applications.

Polycationic agents can be used to deliver nucleic acids to a living subject either intramuscularly, subcutaneously, etc.

The following are examples of useful polypeptides as polycationic agents: polylysine, polyarginine, polyornithine, and protamine. Other examples of useful polypeptides include histones, protamines, human serum albumin, DNA binding proteins, non-histone chromosomal proteins, coat proteins from DNA viruses, such as  $\Phi$ X174, transcriptional factors also contain domains that bind DNA and therefore may be useful as nucleic acid condensing agents. Briefly, transcriptional factors such as C/CEBP, c-jun, c-fos, AP-1, AP-2, AP-3, CPF, Prot-1, Sp-1, Oct-1, Oct-2, CREP, and TFIID contain basic domains that bind DNA sequences.

Organic polycationic agents include: spermine, spermidine, and putrescine.

The dimensions and of the physical properties of a polycationic agent can be extrapolated from the list above, to construct other polypeptide polycationic agents or to produce synthetic polycationic agents.

#### G. Synthetic Polycationic Agents

Synthetic polycationic agents which are useful in pharmaceutical compositions include, for example, DEAE-dextran, polybrene. Lipofectin™, and lipofectAMINE™ are monomers that form polycationic complexes when combined with polynucleotides or polypeptides.

### Immunodiagnostic Assays

*Neisseria* MenB antigens, or antigenic fragments thereof, of the invention can be used in immunoassays to detect antibody levels (or, conversely, anti-*Neisseria* MenB antibodies can be used to detect antigen levels). Immunoassays based on well defined, recombinant antigens can be developed to replace invasive diagnostics methods. Antibodies to *Neisseria* MenB proteins or fragments thereof within biological samples, including for example, blood or serum samples, can be detected. Design of the immunoassays is subject to a great deal of variation, and a variety of these are known in the art. Protocols for the immunoassay may be based, for example, upon competition, or direct reaction, or sandwich type assays. Protocols may also, for example, use solid supports, or may be by immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide; the labels may be, for example, fluorescent, chemiluminescent, radioactive, or dye molecules. Assays which amplify the signals from the probe are also known; examples of which are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

Kits suitable for immunodiagnosis and containing the appropriate labeled reagents are constructed by packaging the appropriate materials, including the compositions of the invention, in suitable containers, along with the remaining reagents and materials (for example, suitable buffers, salt solutions, *etc.*) required for the conduct of the assay, as well as suitable set of assay instructions.

### Nucleic Acid Hybridization

“Hybridization” refers to the association of two nucleic acid sequences to one another by hydrogen bonding. Typically, one sequence will be fixed to a solid support and the other will be free in solution. Then, the two sequences will be placed in contact with one another under conditions that favor hydrogen bonding. Factors that affect this bonding include: the type and volume of solvent; reaction temperature; time of hybridization; agitation; agents to block the non-specific attachment of the liquid phase sequence to the solid support (Denhardt's reagent or BLOTTO); concentration of the sequences; use of compounds to increase the rate of association of sequences (dextran sulfate or polyethylene glycol); and the

stringency of the washing conditions following hybridization. See Sambrook *et al.* (*supra*) Volume 2, chapter 9, pages 9.47 to 9.57.

"Stringency" refers to conditions in a hybridization reaction that favor association of very similar sequences over sequences that differ. For example, the combination of temperature and salt concentration should be chosen that is approximately 120 to 200°C below the calculated  $T_m$  of the hybrid under study. The temperature and salt conditions can often be determined empirically in preliminary experiments in which samples of genomic DNA immobilized on filters are hybridized to the sequence of interest and then washed under conditions of different stringencies. See Sambrook *et al.* at page 9.50.

Variables to consider when performing, for example, a Southern blot are (1) the complexity of the DNA being blotted and (2) the homology between the probe and the sequences being detected. The total amount of the fragment(s) to be studied can vary a magnitude of 10, from 0.1 to 1 µg for a plasmid or phage digest to  $10^{-9}$  to  $10^{-8}$  g for a single copy gene in a highly complex eukaryotic genome. For lower complexity polynucleotides, substantially shorter blotting, hybridization, and exposure times, a smaller amount of starting polynucleotides, and lower specific activity of probes can be used. For example, a single-copy yeast gene can be detected with an exposure time of only 1 hour starting with 1 µg of yeast DNA, blotting for two hours, and hybridizing for 4-8 hours with a probe of  $10^8$  cpm/µg. For a single-copy mammalian gene a conservative approach would start with 10 µg of DNA, blot overnight, and hybridize overnight in the presence of 10% dextran sulfate using a probe of greater than  $10^8$  cpm/µg, resulting in an exposure time of ~24 hours.

Several factors can affect the melting temperature ( $T_m$ ) of a DNA-DNA hybrid between the probe and the fragment of interest, and consequently, the appropriate conditions for hybridization and washing. In many cases the probe is not 100% homologous to the fragment. Other commonly encountered variables include the length and total G+C content of the hybridizing sequences and the ionic strength and formamide content of the hybridization buffer. The effects of all of these factors can be approximated by a single equation:

$$T_m = 81 + 16.6(\log_{10} C_i) + 0.4(\%(G + C)) - 0.6(\%\text{formamide}) - 600/n - 1.5(\%\text{mismatch})$$

where  $C_i$  is the salt concentration (monovalent ions) and  $n$  is the length of the hybrid in base pairs (slightly modified from Meinkoth & Wahl (1984) *Anal. Biochem.* 138:267-284).

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In designing a hybridization experiment, some factors affecting nucleic acid hybridization can be conveniently altered. The temperature of the hybridization and washes and the salt concentration during the washes are the simplest to adjust. As the temperature of the hybridization increases (i.e., stringency), it becomes less likely for hybridization to occur between strands that are nonhomologous, and as a result, background decreases. If the radiolabeled probe is not completely homologous with the immobilized fragment (as is frequently the case in gene family and interspecies hybridization experiments), the hybridization temperature must be reduced, and background will increase. The temperature of the washes affects the intensity of the hybridizing band and the degree of background in a similar manner. The stringency of the washes is also increased with decreasing salt concentrations.

In general, convenient hybridization temperatures in the presence of 50% formamide are 42°C for a probe with is 95% to 100% homologous to the target fragment, 37°C for 90% to 95% homology, and 32°C for 85% to 90% homology. For lower homologies, formamide content should be lowered and temperature adjusted accordingly, using the equation above. If the homology between the probe and the target fragment are not known, the simplest approach is to start with both hybridization and wash conditions which are nonstringent. If non-specific bands or high background are observed after autoradiography, the filter can be washed at high stringency and reexposed. If the time required for exposure makes this approach impractical, several hybridization and/or washing stringencies should be tested in parallel.

#### Nucleic Acid Probe Assays

Methods such as PCR, branched DNA probe assays, or blotting techniques utilizing nucleic acid probes according to the invention can determine the presence of cDNA or mRNA. A probe is said to "hybridize" with a sequence of the invention if it can form a duplex or double stranded complex, which is stable enough to be detected.

The nucleic acid probes will hybridize to the Neisserial nucleotide sequences of the invention (including both sense and antisense strands). Though many different nucleotide sequences will encode the amino acid sequence, the native Neisserial sequence is preferred because it is the actual sequence present in cells. mRNA represents a coding sequence and so

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a probe should be complementary to the coding sequence; single-stranded cDNA is complementary to mRNA, and so a cDNA probe should be complementary to the non-coding sequence.

The probe sequence need not be identical to the Neisserial sequence (or its complement) -- some variation in the sequence and length can lead to increased assay sensitivity if the nucleic acid probe can form a duplex with target nucleotides, which can be detected. Also, the nucleic acid probe can include additional nucleotides to stabilize the formed duplex. Additional Neisserial sequence may also be helpful as a label to detect the formed duplex. For example, a non-complementary nucleotide sequence may be attached to the 5' end of the probe, with the remainder of the probe sequence being complementary to a Neisserial sequence. Alternatively, non-complementary bases or longer sequences can be interspersed into the probe, provided that the probe sequence has sufficient complementarity with the a Neisserial sequence in order to hybridize therewith and thereby form a duplex which can be detected.

The exact length and sequence of the probe will depend on the hybridization conditions, such as temperature, salt condition and the like. For example, for diagnostic applications, depending on the complexity of the analyte sequence, the nucleic acid probe typically contains at least 10-20 nucleotides, preferably 15-25, and more preferably at least 30 nucleotides, although it may be shorter than this. Short primers generally require cooler temperatures to form sufficiently stable hybrid complexes with the template.

Probes may be produced by synthetic procedures, such as the triester method of Matteucci *et al.* (*J. Am. Chem. Soc.* (1981) 103:3185), or according to Urdea *et al.* (*Proc. Natl. Acad. Sci. USA* (1983) 80: 7461), or using commercially available automated oligonucleotide synthesizers.

The chemical nature of the probe can be selected according to preference. For certain applications, DNA or RNA are appropriate. For other applications, modifications may be incorporated e.g., backbone modifications, such as phosphorothioates or methylphosphonates, can be used to increase *in vivo* half-life, alter RNA affinity, increase nuclease resistance *etc.* (e.g., see Agrawal & Iyer (1995) *Curr Opin Biotechnol* 6:12-19; Agrawal (1996) *TIBTECH* 14:376-387); analogues such as peptide nucleic acids may also be

used (e.g., see Corey (1997) *TIBTECH* 15:224-229; Buchardt *et al.* (1993) *TIBTECH* 11:384-386).

One example of a nucleotide hybridization assay is described by Urdea *et al.* in international patent application WO92/02526 (see also U.S. Patent 5,124,246).

Alternatively, the polymerase chain reaction (PCR) is another well-known means for detecting small amounts of target nucleic acids. The assay is described in: Mullis *et al.* (*Meth. Enzymol.* (1987) 155: 335-350); US patent 4,683,195; and US patent 4,683,202. Two "primer" nucleotides hybridize with the target nucleic acids and are used to prime the reaction. The primers can comprise sequence that does not hybridize to the sequence of the amplification target (or its complement) to aid with duplex stability or, for example, to incorporate a convenient restriction site. Typically, such sequence will flank the desired *Neisseria* sequence.

A thermostable polymerase creates copies of target nucleic acids from the primers using the original target nucleic acids as a template. After a threshold amount of target nucleic acids are generated by the polymerase, they can be detected by more traditional methods, such as Southern blots. When using the Southern blot method, the labeled probe will hybridize to the *Neisseria* sequence (or its complement).

Also, mRNA or cDNA can be detected by traditional blotting techniques described in Sambrook *et al.* (*supra*). mRNA, or cDNA generated from mRNA using a polymerase enzyme, can be purified and separated using gel electrophoresis. The nucleic acids on the gel are then blotted onto a solid support, such as nitrocellulose. The solid support is exposed to a labeled probe and then washed to remove any unhybridized probe. Next, the duplexes containing the labeled probe are detected. Typically, the probe is labeled with a radioactive moiety.

## EXAMPLES

The invention is based on the 961 nucleotide sequences from the genome of *N. meningitidis* set out in Appendix C, SEQ ID NOs:1-961 of the '573 application, which together represent substantially the complete genome of serotype B of *N. meningitidis*, as well as the full length genome sequence shown in Appendix D, SEQ ID NO 1068 of the '573

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application, and the full length genome sequence shown in Appendix A hereto, SEQ ID NO. 1.

It will be self-evident to the skilled person how this sequence information can be utilized according to the invention, as above described.

The standard techniques and procedures which may be employed in order to perform the invention (e.g. to utilize the disclosed sequences to predict polypeptides useful for vaccination or diagnostic purposes) were summarized above. This summary is not a limitation on the invention but, rather, gives examples that may be used, but are not required.

These sequences are derived from contigs shown in Appendix C (SEQ ID NOs 1-961) and from the full length genome sequence shown in Appendix D (SEQ ID NO 1068), which were prepared during the sequencing of the genome of *N. meningitidis* (strain B). The full length sequence was assembled using the TIGR Assembler as described by G.S. Sutton et al., *TIGR Assembler: A New Tool for Assembling Large Shotgun Sequencing Projects*, Genome Science and Technology, 1:9-19 (1995) [see also R. D. Fleischmann, et al., Science 269, 496-512 (1995); C. M. Fraser, et al., Science 270, 397-403 (1995); C. J. Bult, et al., Science 273, 1058-73 (1996); C. M. Fraser, et al., Nature 390, 580-586 (1997); J.-F. Tomb, et al., Nature 388, 539-547 (1997); H. P. Klenk, et al., Nature 390, 364-70 (1997); C. M. Fraser, et al., Science 281, 375-88 (1998); M. J. Gardner, et al., Science 282, 1126-1132 (1998); K. E. Nelson, et al., Nature 399, 323-9 (1999)]. Then, using the above-described methods, putative translation products of the sequences were determined. Computer analysis of the translation products were determined based on database comparisons. Corresponding gene and protein sequences, if any, were identified in *Neisseria meningitidis* (Strain A) and *Neisseria gonorrhoeae*. Then the proteins were expressed, purified, and characterized to assess their antigenicity and immunogenicity.

In particular, the following methods were used to express, purify, and biochemically characterize the proteins of the invention.

#### Chromosomal DNA Preparation

*N. meningitidis* strain 2996 was grown to exponential phase in 100 ml of GC medium, harvested by centrifugation, and resuspended in 5 ml buffer (20% Sucrose, 50 mM Tris-HCl, 50 mM EDTA, adjusted to pH 8.0). After 10 minutes incubation on ice, the bacteria were



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lysed by adding 10 ml lysis solution (50 mM NaCl, 1% Na-Sarkosyl, 50 µg/ml Proteinase K), and the suspension was incubated at 37°C for 2 hours. Two phenol extractions (equilibrated to pH 8) and one  $\text{CHCl}_3$ /isoamylalcohol (24:1) extraction were performed. DNA was precipitated by addition of 0.3M sodium acetate and 2 volumes ethanol, and was collected by centrifugation. The pellet was washed once with 70% ethanol and redissolved in 4 ml buffer (10 mM Tris-HCl, 1mM EDTA, pH 8). The DNA concentration was measured by reading the OD at 260 nm.

### Oligonucleotide design

Synthetic oligonucleotide primers were designed on the basis of the coding sequence of each ORF, using (a) the meningococcus B sequence when available, or (b) the gonococcus/meningococcus A sequence, adapted to the codon preference usage of meningococcus. Any predicted signal peptides were omitted, by deducing the 5'-end amplification primer sequence immediately downstream from the predicted leader sequence.

For most ORFs, the 5' primers included two restriction enzyme recognition sites (*Bam*HI-*Nde*I, *Bam*HI-*Nhe*I, or *Eco*RI-*Nhe*I, depending on the gene's restriction pattern); the 3' primers included a *Xho*I restriction site. This procedure was established in order to direct the cloning of each amplification product (corresponding to each ORF) into two different expression systems: pGEX-KG (using either *Bam*HI-*Xho*I or *Eco*RI-*Xho*I), and pET21b+ (using either *Nde*I-*Xho*I or *Nhe*I-*Xho*I).

5'-end primer tail:	<u>CGCGGATCCCATATG</u>	( <i>Bam</i> HI- <i>Nde</i> I)
	<u>CGCGGATCCGCTAGC</u>	( <i>Bam</i> HI- <i>Nhe</i> I)
	<u>CCGGAATTCTAGCTAGC</u>	( <i>Eco</i> RI- <i>Nhe</i> I)
3'-end primer tail:	<u>CCCGCTCGAG</u>	( <i>Xho</i> I)

For some ORFs, two different amplifications were performed to clone each ORF in the two expression systems. Two different 5' primers were used for each ORF; the same 3' *Xho*I primer was used as before:

5'-end primer tail:	<u>GGAATTCCATATGGCCATGG</u>	( <i>Nde</i> I)
5'-end primer tail:	<u>CGGGATCC</u>	( <i>Bam</i> HI)

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Other ORFs were cloned in the pTRC expression vector and expressed as an amino-terminus His-tag fusion. The predicted signal peptide may be included in the final product. *NheI*-*BamHI* restriction sites were incorporated using primers:

5'-end primer tail: GATCAGCTAGCCATATG (*NheI*)

3'-end primer tail: CGGGATCC (*BamHI*)

As well as containing the restriction enzyme recognition sequences, the primers included nucleotides which hybridized to the sequence to be amplified. The number of hybridizing nucleotides depended on the melting temperature of the whole primer, and was determined for each primer using the formulae:

$$T_m = 4 (G+C) + 2 (A+T) \quad (\text{tail excluded})$$

$$T_m = 64.9 + 0.41 (\% \text{ GC}) - 600/N \quad (\text{whole primer})$$

The average melting temperature of the selected oligos were 65-70°C for the whole oligo and 50-55°C for the hybridising region alone.

Oligos were synthesized by a Perkin Elmer 394 DNA/RNA Synthesizer, eluted from the columns in 2 ml  $\text{NH}_4\text{-OH}$ , and deprotected by 5 hours incubation at 56 °C. The oligos were precipitated by addition of 0.3M Na-Acetate and 2 volumes ethanol. The samples were then centrifuged and the pellets resuspended in either 100µl or 1ml of water.  $\text{OD}_{260}$  was determined using a Perkin Elmer Lambda Bio spectrophotometer and the concentration was determined and adjusted to 2-10 pmol/µl.

Table 1 shows the forward and reverse primers used for each amplification. In certain cases, it might be noted that the sequence of the primer does not exactly match the sequence in the ORF. When initial amplifications are performed, the complete 5' and/or 3' sequence may not be known for some meningococcal ORFs, although the corresponding sequences may have been identified in gonococcus. For amplification, the gonococcal sequences could thus be used as the basis for primer design, altered to take account of codon preference. In particular, the following codons may be changed: ATA→ATT; TCG→TCT; CAG→CAA; AAG→AAA; GAG→GAA; CGA and CGG→CGC; GGG→GGC.

### Amplification

The standard PCR protocol was as follows: 50-200 ng of genomic DNA were used as a template in the presence of 20-40 µM of each oligo, 400-800 µM dNTPs solution, 1x PCR

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buffer (including 1.5 mM MgCl<sub>2</sub>), 2.5 units *TaqI* DNA polymerase (using Perkin-Elmer AmpliTaq, GIBCO Platinum, Pwo DNA polymerase, or Tahara Shuzo Taq polymerase).

In some cases, PCR was optimised by the addition of 10µl DMSO or 50 µl 2M betaine.

After a hot start (adding the polymerase during a preliminary 3 minute incubation of the whole mix at 95°C), each sample underwent a double-step amplification: the first 5 cycles were performed using as the hybridization temperature the one of the oligos excluding the restriction enzymes tail, followed by 30 cycles performed according to the hybridization temperature of the whole length oligos. The cycles were followed by a final 10 minute extension step at 72°C.

The standard cycles were as follows:

	Denaturation	Hybridisation	Elongation
First 5 cycles	30 seconds 95°C	30 seconds 50-55°C	30-60 seconds 72°C
Last 30 cycles	30 seconds 95°C	30 seconds 65-70°C	30-60 seconds 72°C

The elongation time varied according to the length of the ORF to be amplified.

The amplifications were performed using either a 9600 or a 2400 Perkin Elmer GeneAmp PCR System. To check the results, 1/10 of the amplification volume was loaded onto a 1-1.5% agarose gel and the size of each amplified fragment compared with a DNA molecular weight marker.

The amplified DNA was either loaded directly on a 1% agarose gel or first precipitated with ethanol and resuspended in a suitable volume to be loaded on a 1% agarose gel. The DNA fragment corresponding to the right size band was then eluted and purified from gel, using the Qiagen Gel Extraction Kit, following the instructions of the manufacturer. The final volume of the DNA fragment was 30µl or 50µl of either water or 10mM Tris, pH 8.5.

#### **Digestion of PCR fragments**

The purified DNA corresponding to the amplified fragment was split into 2 aliquots and double-digested with:

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*NdeI/XhoI* or *NheI/XhoI* for cloning into pET-21b+ and further expression of the protein as a C-terminus His-tag fusion

*BamHI/XhoI* or *EcoRI/XhoI* for cloning into pGEX-KG and further expression of the protein as a GST N-terminus fusion.

For ORF 76, *NheI/BamHI* for cloning into pTRC-HisA vector and further expression of the protein as N-terminus His-tag fusion.

Each purified DNA fragment was incubated (37°C for 3 hours to overnight) with 20 units of each restriction enzyme (New England Biolabs ) in a either 30 or 40 µl final volume in the presence of the appropriate buffer. The digestion product was then purified using the QIAquick PCR purification kit, following the manufacturer's instructions, and eluted in a final volume of 30 (or 50) µl of either water or 10mM Tris-HCl, pH 8.5. The final DNA concentration was determined by 1% agarose gel electrophoresis in the presence of titrated molecular weight marker.

#### **Digestion of the cloning vectors (pET22B, pGEX-KG and pTRC-His A)**

10 µg plasmid was double-digested with 50 units of each restriction enzyme in 200 µl reaction volume in the presence of appropriate buffer by overnight incubation at 37°C. After loading the whole digestion on a 1% agarose gel, the band corresponding to the digested vector was purified from the gel using the Qiagen QIAquick Gel Extraction Kit and the DNA was eluted in 50 µl of 10 mM Tris-HCl, pH 8.5. The DNA concentration was evaluated by measuring OD<sub>260</sub> of the sample, and adjusted to 50 µg/µl. 1 µl of plasmid was used for each cloning procedure.

#### **Cloning**

The fragments corresponding to each ORF, previously digested and purified, were ligated in both pET22b and pGEX-KG. In a final volume of 20 µl, a molar ratio of 3:1 fragment/vector was ligated using 0.5 µl of NEB T4 DNA ligase (400 units/µl), in the presence of the buffer supplied by the manufacturer. The reaction was incubated at room temperature for 3 hours. In some experiments, ligation was performed using the Boehringer "Rapid Ligation Kit", following the manufacturer's instructions.

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In order to introduce the recombinant plasmid in a suitable strain, 100  $\mu$ l *E. coli* DH5 competent cells were incubated with the ligase reaction solution for 40 minutes on ice, then at 37°C for 3 minutes, then, after adding 800  $\mu$ l LB broth, again at 37°C for 20 minutes. The cells were then centrifuged at maximum speed in an Eppendorf microfuge and resuspended in approximately 200  $\mu$ l of the supernatant. The suspension was then plated on LB ampicillin (100 mg/ml).

The screening of the recombinant clones was performed by growing 5 randomly-chosen colonies overnight at 37 °C in either 2 ml (pGEX or pTC clones) or 5ml (pET clones) LB broth + 100  $\mu$ g/ml ampicillin. The cells were then pelleted and the DNA extracted using the Qiagen QIAprep Spin Miniprep Kit, following the manufacturer's instructions, to a final volume of 30  $\mu$ l. 5  $\mu$ l of each individual miniprep (approximately 1g) were digested with either *NdeI/XhoI* or *BamHI/XhoI* and the whole digestion loaded onto a 1-1.5% agarose gel (depending on the expected insert size), in parallel with the molecular weight marker (1Kb DNA Ladder, GIBCO). The screening of the positive clones was made on the base of the correct insert size.

### **Cloning**

Certain ORFs may be cloned into the pGEX-HIS vector using *EcoRI-PstI*, *EcoRI-SalI*, or *SalI-PstI* cloning sites. After cloning, the recombinant plasmids may be introduced in the *E.coli* host W3110.

### **Expression**

Each ORF cloned into the expression vector may then be transformed into the strain suitable for expression of the recombinant protein product. 1  $\mu$ l of each construct was used to transform 30  $\mu$ l of *E.coli* BL21 (pGEX vector), *E.coli* TOP 10 (pTRC vector) or *E.coli* BL21-DE3 (pET vector), as described above. In the case of the pGEX-His vector, the same *E.coli* strain (W3110) was used for initial cloning and expression. Single recombinant colonies were inoculated into 2ml LB+Amp (100  $\mu$ g/ml), incubated at 37°C overnight, then diluted 1:30 in 20 ml of LB+Amp (100  $\mu$ g/ml) in 100 ml flasks, making sure that the OD<sub>600</sub> ranged between 0.1 and 0.15. The flasks were incubated at 30°C into gyratory water bath shakers until OD indicated exponential growth suitable for induction of expression (0.4-0.8 OD for

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pET and pTRC vectors; 0.8-1 OD for pGEX and pGEX-His vectors). For the pET, pTRC and pGEX-His vectors, the protein expression was induced by addition of 1mM IPTG, whereas in the case of pGEX system the final concentration of IPTG was 0.2 mM. After 3 hours incubation at 30°C, the final concentration of the sample was checked by OD. In order to check expression, 1ml of each sample was removed, centrifuged in a microfuge, the pellet resuspended in PBS, and analysed by 12% SDS-PAGE with Coomassie Blue staining. The whole sample was centrifuged at 6000g and the pellet resuspended in PBS for further use.

#### **GST-fusion proteins large-scale purification.**

A single colony was grown overnight at 37°C on LB+Amp agar plate. The bacteria were inoculated into 20 ml of LB+Amp liquid culture in a water bath shaker and grown overnight. Bacteria were diluted 1:30 into 600 ml of fresh medium and allowed to grow at the optimal temperature (20-37°C) to OD<sub>550</sub> 0.8-1. Protein expression was induced with 0.2mM IPTG followed by three hours incubation. The culture was centrifuged at 8000 rpm at 4°C. The supernatant was discarded and the bacterial pellet was resuspended in 7.5 ml cold PBS. The cells were disrupted by sonication on ice for 30 sec at 40W using a Branson sonifier B-15, frozen and thawed two times and centrifuged again. The supernatant was collected and mixed with 150µl Glutathione-Sepharose 4B resin (Pharmacia) (previously washed with PBS) and incubated at room temperature for 30 minutes. The sample was centrifuged at 700g for 5 minutes at 4°C. The resin was washed twice with 10 ml cold PBS for 10 minutes, resuspended in 1ml cold PBS, and loaded on a disposable column. The resin was washed twice with 2ml cold PBS until the flow-through reached OD<sub>280</sub> of 0.02-0.06. The GST-fusion protein was eluted by addition of 700µl cold Glutathione elution buffer (10mM reduced glutathione, 50mM Tris-HCl) and fractions collected until the OD<sub>280</sub> was 0.1. 21µl of each fraction were loaded on a 12% SDS gel using either Biorad SDS-PAGE Molecular weight standard broad range (M1) (200, 116.25, 97.4, 66.2, 45, 31, 21.5, 14.4, 6.5 kDa) or Amersham Rainbow Marker (M') (220, 66, 46, 30, 21.5, 14.3 kDa) as standards. As the MW of GST is 26kDa, this value must be added to the MW of each GST-fusion protein.

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**His-fusion soluble proteins large-scale purification.**

A single colony was grown overnight at 37°C on a LB + Amp agar plate. The bacteria were inoculated into 20ml of LB+Amp liquid culture and incubated overnight in a water bath shaker. Bacteria were diluted 1:30 into 600ml fresh medium and allowed to grow at the optimal temperature (20-37°C) to OD<sub>550</sub> 0.6-0.8. Protein expression was induced by addition of 1 mM IPTG and the culture further incubated for three hours. The culture was centrifuged at 8000 rpm at 4°C, the supernatant was discarded and the bacterial pellet was resuspended in 7.5ml cold 10mM imidazole buffer (300 mM NaCl, 50 mM phosphate buffer, 10 mM imidazole, pH 8). The cells were disrupted by sonication on ice for 30 sec at 40W using a Branson sonifier B-15, frozen and thawed two times and centrifuged again. The supernatant was collected and mixed with 150µl Ni<sup>2+</sup>-resin (Pharmacia) (previously washed with 10mM imidazole buffer) and incubated at room temperature with gentle agitation for 30 minutes. The sample was centrifuged at 700g for 5 minutes at 4°C. The resin was washed twice with 10 ml cold 10mM imidazole buffer for 10 minutes, resuspended in 1ml cold 10mM imidazole buffer and loaded on a disposable column. The resin was washed at 4°C with 2ml cold 10mM imidazole buffer until the flow-through reached the O.D<sub>280</sub> of 0.02-0.06. The resin was washed with 2ml cold 20mM imidazole buffer (300 mM NaCl, 50 mM phosphate buffer, 20 mM imidazole, pH 8) until the flow-through reached the O.D<sub>280</sub> of 0.02-0.06. The His-fusion protein was eluted by addition of 700µl cold 250mM imidazole buffer (300 mM NaCl, 50 mM phosphate buffer, 250 mM imidazole, pH 8) and fractions collected until the O.D<sub>280</sub> was 0.1. 21µl of each fraction were loaded on a 12% SDS gel.

**His-fusion insoluble proteins large-scale purification.**

A single colony was grown overnight at 37 °C on a LB + Amp agar plate. The bacteria were inoculated into 20 ml of LB+Amp liquid culture in a water bath shaker and grown overnight. Bacteria were diluted 1:30 into 600ml fresh medium and let to grow at the optimal temperature (37°C) to O.D<sub>550</sub> 0.6-0.8. Protein expression was induced by addition of 1 mM IPTG and the culture further incubated for three hours. The culture was centrifuged at 8000rpm at 4°C. The supernatant was discarded and the bacterial pellet was resuspended in 7.5 ml buffer B (urea 8M, 10mM Tris-HCl, 100mM phosphate buffer, pH 8.8). The cells were disrupted by sonication on ice for 30 sec at 40W using a Branson sonifier B-15, frozen

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and thawed twice and centrifuged again. The supernatant was stored at -20°C, while the pellets were resuspended in 2 ml guanidine buffer (6M guanidine hydrochloride, 100mM phosphate buffer, 10 mM Tris-HCl, pH 7.5) and treated in a homogenizer for 10 cycles. The product was centrifuged at 13000 rpm for 40 minutes. The supernatant was mixed with 150µl Ni<sup>2+</sup>-resin (Pharmacia) (previously washed with buffer B) and incubated at room temperature with gentle agitation for 30 minutes. The sample was centrifuged at 700 g for 5 minutes at 4°C. The resin was washed twice with 10 ml buffer B for 10 minutes, resuspended in 1ml buffer B, and loaded on a disposable column. The resin was washed at room temperature with 2ml buffer B until the flow-through reached the OD<sub>280</sub> of 0.02-0.06. The resin was washed with 2ml buffer C (urea 8M, 10mM Tris-HCl, 100mM phosphate buffer, pH 6.3) until the flow-through reached the O.D<sub>280</sub> of 0.02-0.06. The His-fusion protein was eluted by addition of 700µl elution buffer (urea 8M, 10mM Tris-HCl, 100mM phosphate buffer, pH 4.5) and fractions collected until the OD<sub>280</sub> was 0.1. 21µl of each fraction were loaded on a 12% SDS gel.

#### **His-fusion proteins renaturation**

10% glycerol was added to the denatured proteins. The proteins were then diluted to 20µg/ml using dialysis buffer I (10% glycerol, 0.5M arginine, 50mM phosphate buffer, 5mM reduced glutathione, 0.5mM oxidised glutathione, 2M urea, pH 8.8) and dialysed against the same buffer at 4°C for 12-14 hours. The protein was further dialysed against dialysis buffer II (10% glycerol, 0.5M arginine, 50mM phosphate buffer, 5mM reduced glutathione, 0.5mM oxidised glutathione, pH 8.8) for 12-14 hours at 4°C. Protein concentration was evaluated using the formula:

$$\text{Protein (mg/ml)} = (1.55 \times \text{OD}_{280}) - (0.76 \times \text{OD}_{260})$$

#### **Mice immunisations**

20µg of each purified protein were used to immunise mice intraperitoneally. In the case of some ORFs, Balb-C mice were immunised with Al(OH)<sub>3</sub> as adjuvant on days 1, 21 and 42, and immune response was monitored in samples taken on day 56. For other ORFs, CD1 mice could be immunised using the same protocol. For other ORFs, CD1 mice could be immunised using Freund's adjuvant, and the same immunisation protocol was used, except that the immune response was measured on day 42, rather than 56. Similarly, for still other



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ORFs, CD1 mice could be immunised with Freund's adjuvant, but the immune response was measured on day 49.

#### **ELISA assay (sera analysis)**

The acapsulated MenB M7 strain was plated on chocolate agar plates and incubated overnight at 37°C. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into 7ml of Mueller-Hinton Broth (Difco) containing 0.25% Glucose. Bacterial growth was monitored every 30 minutes by following OD<sub>620</sub>. The bacteria were let to grow until the OD reached the value of 0.3-0.4. The culture was centrifuged for 10 minutes at 10000 rpm. The supernatant was discarded and bacteria were washed once with PBS, resuspended in PBS containing 0.025% formaldehyde, and incubated for 2 hours at room temperature and then overnight at 4°C with stirring. 100µl bacterial cells were added to each well of a 96 well Greiner plate and incubated overnight at 4°C. The wells were then washed three times with PBT washing buffer (0.1% Tween-20 in PBS). 200 µl of saturation buffer (2.7% Polyvinylpyrrolidone 10 in water) was added to each well and the plates incubated for 2 hours at 37°C. Wells were washed three times with PBT. 200 µl of diluted sera (Dilution buffer: 1% BSA, 0.1% Tween-20, 0.1% NaN<sub>3</sub> in PBS) were added to each well and the plates incubated for 90 minutes at 37°C. Wells were washed three times with PBT. 100 µl of HRP-conjugated rabbit anti-mouse (Dako) serum diluted 1:2000 in dilution buffer were added to each well and the plates were incubated for 90 minutes at 37°C. Wells were washed three times with PBT buffer. 100 µl of substrate buffer for HRP (25 ml of citrate buffer pH5, 10 mg of O-phenildiamine and 10 µl of H<sub>2</sub>O) were added to each well and the plates were left at room temperature for 20 minutes. 100 µl H<sub>2</sub>SO<sub>4</sub> was added to each well and OD<sub>490</sub> was followed. The ELISA was considered positive when OD<sub>490</sub> was 2.5 times the respective pre-immune sera.

#### **FACScan bacteria Binding Assay procedure.**

The acapsulated MenB M7 strain was plated on chocolate agar plates and incubated overnight at 37°C. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into 4 tubes containing 8ml each Mueller-Hinton Broth (Difco) containing 0.25% glucose. Bacterial growth was monitored every 30 minutes by following

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OD<sub>620</sub>. The bacteria were let to grow until the OD reached the value of 0.35-0.5. The culture was centrifuged for 10 minutes at 4000 rpm. The supernatant was discarded and the pellet was resuspended in blocking buffer (1% BSA, 0.4% NaN<sub>3</sub>) and centrifuged for 5 minutes at 4000 rpm. Cells were resuspended in blocking buffer to reach OD<sub>620</sub> of 0.07. 100µl bacterial cells were added to each well of a Costar 96 well plate. 100µl of diluted (1:200) sera (in blocking buffer) were added to each well and plates incubated for 2 hours at 4°C. Cells were centrifuged for 5 minutes at 4000 rpm, the supernatant aspirated and cells washed by addition of 200µl/well of blocking buffer in each well. 100µl of R-Phicoerytrin conjugated F(ab)<sub>2</sub> goat anti-mouse, diluted 1:100, was added to each well and plates incubated for 1 hour at 4°C. Cells were spun down by centrifugation at 4000rpm for 5 minutes and washed by addition of 200µl/well of blocking buffer. The supernatant was aspirated and cells resuspended in 200µl/well of PBS, 0.25% formaldehyde. Samples were transferred to FACScan tubes and read. The condition for FACScan setting were: FL1 on, FL2 and FL3 off; FSC-H Treshold:92; FSC PMT Voltage: E 02; SSC PMT: 474; Amp. Gains 7.1; FL-2 PMT: 539. Compensation values: 0.

### **OMV preparations**

Bacteria were grown overnight on 5 GC plates, harvested with a loop and resuspended in 10 ml 20mM Tris-HCl. Heat inactivation was performed at 56°C for 30 minutes and the bacteria disrupted by sonication for 10' on ice ( 50% duty cycle, 50% output ). Unbroken cells were removed by centrifugation at 5000g for 10 minutes and the total cell envelope fraction recovered by centrifugation at 50000g at 4°C for 75 minutes. To extract cytoplasmic membrane proteins from the crude outer membranes, the whole fraction was resuspended in 2% sarkosyl (Sigma) and incubated at room temperature for 20 minutes. The suspension was centrifuged at 10000g for 10 minutes to remove aggregates, and the supernatant further ultracentrifuged at 50000g for 75 minutes to pellet the outer membranes. The outer membranes were resuspended in 10mM Tris-HCl, pH8 and the protein concentration measured by the Bio-Rad Protein assay, using BSA as a standard.

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**Whole Extracts preparation**

Bacteria were grown overnight on a GC plate, harvested with a loop and resuspended in 1ml of 20mM Tris-HCl. Heat inactivation was performed at 56°C for 30' minutes.

**Western blotting**

Purified proteins (500ng/lane), outer membrane vesicles (5 µg) and total cell extracts (25µg) derived from MenB strain 2996 were loaded on 15% SDS-PAGE and transferred to a nitrocellulose membrane. The transfer was performed for 2 hours at 150mA at 4°C, in transferring buffer (0.3 % Tris base, 1.44 % glycine, 20% methanol). The membrane was saturated by overnight incubation at 4°C in saturation buffer (10% skimmed milk, 0.1% Triton X100 in PBS). The membrane was washed twice with washing buffer (3% skimmed milk, 0.1% Triton X100 in PBS) and incubated for 2 hours at 37°C with 1:200 mice sera diluted in washing buffer. The membrane was washed twice and incubated for 90 minutes with a 1:2000 dilution of horseradish peroxidase labeled anti-mouse Ig. The membrane was washed twice with 0.1% Triton X100 in PBS and developed with the Opti-4CN Substrate Kit (Bio-Rad). The reaction was stopped by adding water.

**Bactericidal assay**

MC58 strain was grown overnight at 37°C on chocolate agar plates. 5-7 colonies were collected and used to inoculate 7ml Mueller-Hinton broth. The suspension was incubated at 37°C on a nutator and let to grow until OD<sub>620</sub> was in between 0.5-0.8. The culture was aliquoted into sterile 1.5ml Eppendorf tubes and centrifuged for 20 minutes at maximum speed in a microfuge. The pellet was washed once in Gey's buffer (Gibco) and resuspended in the same buffer to an OD<sub>620</sub> of 0.5, diluted 1:20000 in Gey's buffer and stored at 25°C.

50µl of Gey's buffer/1% BSA was added to each well of a 96-well tissue culture plate. 25µl of diluted (1:100) mice sera (dilution buffer: Gey's buffer/0.2% BSA) were added to each well and the plate incubated at 4°C. 25µl of the previously described bacterial suspension were added to each well. 25µl of either heat-inactivated (56°C waterbath for 30 minutes) or normal baby rabbit complement were added to each well. Immediately after the addition of the baby rabbit complement, 22µl of each sample/well were plated on Mueller-

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Hinton agar plates (time 0). The 96-well plate was incubated for 1 hour at 37°C with rotation and then 22µl of each sample/well were plated on Mueller-Hinton agar plates (time 1). After overnight incubation the colonies corresponding to time 0 and time 1h were counted.

The following DNA and amino acid sequences are identified by titles of the following form: [g, m, or a] [#].[seq or pep], where "g" means a sequence from *N. gonorrhoeae*, "m" means a sequence from *N. meningitidis B*, and "a" means a sequence from *N. meningitidis A*; "#" means the number of the sequence; "seq" means a DNA sequence, and "pep" means an amino acid sequence. For example, "g001.seq" refers to an *N. gonorrhoeae* DNA sequence, number 1. The presence of the suffix "-1" or "-2" to these sequences indicates an additional sequence found for the same ORF. Further, open reading frames are identified as ORF #, where "#" means the number of the ORF, corresponding to the number of the sequence which encodes the ORF, and the ORF designations may be suffixed with ".ng" or ".a", indicating that the ORF corresponds to a *N. gonorrhoeae* sequence or a *N. meningitidis A* sequence, respectively. Computer analysis was performed for the comparisons that follow between "g", "m", and "a" peptide sequences; and therein the "pep" suffix is implied where not expressly stated.

### EXAMPLE 1

The following ORFs were predicted from the contig sequences and/or the full length sequences using the methods herein described.

#### Localization of the ORFs

ORF:            contig:

279            gnm4.seq

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 2>:

m279.seq

```

1   ATAACGCGGA TTTGCGGCTG CTTGATTTC ACGGTTTTC GGGCTTCGGC
51  AAGTTTGTCT GCGGCGGGTT TCATCAGGCT GCAATGGGAA GGTACGGACA
101 CGGGCAGCGG CAGGGCGCGT TTGGCACCGG CTTCTTTGGC GGCAGCCATG
151 GCGCGTCCGA CGGCGGCGGC GTTGCTGCA ATCAGCATTT GTCCGGGTGA
201 GTTGAAGTTG ACGGCTTCGA CCACTTCGCT TTGGGCGGCT TCGGCACAAA
251 TGGCTTTAAC CTGCTCATCT TCCAAGCCGA GAATCGCCGC CATTGCGCCC
301 ACGCCTTGCG GTACGGCGGA CTGCATCAGT TCGGCGCGCA GGCGCACGAG
351 TTTGACCGCG TCGGCAAAAT TCAATGCGCC GGCGGCAACG AGTGCGGTGT
401 ATTCGCCGAG GCTGTGTCCG GCAACGGCGG CAGGCGTTTT GCCGCCCGCT
451 TCTAAATAG

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```

1  ITRICGCLIS TVFRASASLS AAGFIRLOWE GTDTGSGRAR LAPASLAAAM
51  ARPTAAALPA ITICPGELKL TASTTSLWAA SAQMALTCSS SKPRIAAIAP
101 TPCGTADCIS SARRRTSLTA SAKFNAPAAT SAVYSPRLCP ATAAGVLPPA
151 SK*

```

**g279.veq**

1	atgacgcgga	tttgcggctg	cttgatttca	acggttttga	gtgtttcggc
51	aagtttgtcg	gcggcggggt	tcatacaggct	gcaatgggaa	ggaacggata
101	ccggcagcgg	cagggcgcgt	ttggctccgg	cttctttggc	ggcagccatg
151	gtgcgtccga	cggcggcggc	gttgcttgca	atcacgactt	gtccgggcga
201	gttgaagtgt	acggcttcga	ccacttcgcc	ctgtgcggat	tccgcacaaa
251	tctgcctgac	ctgttcatct	tccaaaccca	aaatggccgc	cattggcctt
301	acgccttgcg	gtacggcgga	ctgpatcagt	tccgcgcgca	ggcggacgag
351	tttagcggca	tccgcaaaaat	ccaatgcttc	ggcggcgaca	agcgcggtgt
401	attcgcgcag	gctgtgtccg	gcaacggcgg	caggcgtttt	gccgccactt
451	tccaaataq				

**g279.ppe**

```

1  MTRICGCLIS TVLSVSASLS AAGFIRLOWE GTDTGSGRAR LAPASLAAAM
51  VRPTAAALPA ITTCPGELKL TASTTSPCAD SAQICLTCS SKPKMAAIAP
101 TPCGTADCIS SARRRTSLTA SAKSNSAAT SAVYSPRLCP ATAAGVLPPT
151 SK*

```

```

      10      20      30      40      50      60
m279.pep  ITRICGCLISTVFRASASLSAAGFIRLQWEGTDTGSGRRLAPASLAAAMARPTAAALPA
          :|||||: :|||||:|||||:|||||:|||||:|||||:|||||:|||||:
g279      MTRICGCLISTVLSVSASLSAAGFIRLQWEGTDTGSGRRLAPASLAAAMVRPTAAALPA
          10      20      30      40      50      60

      70      80      90      100     110     120
m279.pep  ITICPGELKLTASTTSLWAASQMALTCSSSKPRIAAIAPTPCGTADCISSARRRTSLTA
          |||||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
g279      ITTCPGELKLTASTTSPCADSAQICLTCSSSKPKMAAIAPTPCGTADCISSARRRTSLTA
          70      80      90      100     110     120

      130     140     150
m279.pep  SAKFNAPAATSAVYSPRLCPATAAGVLPPTSIX
          |||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
g279      SAKSNASAATSAVYSPRLCPATAAGVLPPTSIX
          130     140     150

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a279.seq

1	ATGACNCNGA	TTTGCGGCTG	CTTGATTCA	ACGGTTTNNNA	GGGCTTCGGC
51	GAGTTTGTCG	GCGGCGGGTT	TCATGAGGCT	GCAATGGGAA	GGTACNGACA
101	CNGGCAGCGG	CAGGGCGCGT	TTGGCGCCGG	CTTCTTTGGC	GGCAAGCATA
151	GCGCGCTCGA	CGGCGGCGGC	ATTGCTGCA	ATCAGACTT	GTCCGGGCGA
201	TTTGAAGTTG	ACGGCTTCAA	CCACTTCATC	CTGTGCGGAT	TCGGCGCAA
251	GGTGTTTTAC	CTGTTCATCT	TCCAAGCCGA	GAATCGCCG	CATTGCGCCG
301	ACGCCTTGCG	GTACGGCGGA	TCCCATCAGT	TCGGCGCGCA	NGGCGACGAC
351	TTTGACCGCG	TOGGCAAAT	CCAATGCGCC	GGCGGCAACN	AGTGCGGTGT

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401 ATTCGCCGAN GCTGTGTCCG GCAACGGCGG CAGGCGTTTT GCCGCCCGCT  
451 TCCGAATAG

This corresponds to the amino acid sequence <SEQ ID 7; ORF 279.a>:

a279.pep  
1 MTXICGCLIS TVXRASASLS AAGFMRLQWE GTDTGSGRAR LAPASLAASI  
51 ARSTAAALPA ITTCPGELKL TASTTSSCAD SAQICFTCSS SKPRIAAIAP  
101 TPCGTADCIS SARXRTSLTA SAKSNAPAAT SAVYSPXLCP ATAAGVLPPA  
151 SE\*

m279/a279 ORFs 279 and 279.a showed a 88.2% identity in 152 aa overlap

	10	20	30	40	50	60
m279.pep	ITRICGCLISTVFRASASLSAAGFIRLQWEGTDTGSGRARLAPASLAAAMARPTAAALPA					
	:              :					
a279	MTXICGCLISTVXRASASLSAAGFMRLQWEGTDTGSGRARLAPASLAASIARSTAAALPA					
	10	20	30	40	50	60
	70	80	90	100	110	120
m279.pep	ITICPGELKLTA TTTSLWAASAQMALTCSSSKPRIAAIAPTPCGTADCIS SARRTSLTA					
	:					
a279	ITTCPGELKLTA TTTSSCADSAQICFTCSSSKPRIAAIAPTPCGTADCIS SARXRTSLTA					
	70	80	90	100	110	120
	130	140	150			
m279.pep	SAKFNAPAATS AVYSPRLCPATAAGVLPPASKX					
a279	SAKSNAPAATS AVYSPXLCPATAAGVLPPASEX					
	130	140	150			

519 and 519-1 gnm7.seq

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 8>:

m519.seq (partial)  
1 ..TCCGTTATCG GCGTATGGA GTTGGACAAA ACGTTTGAAG AACGCGACGA  
51 AATCAACAGT ACTGTTGTTG CGGCTTTGGA CGAGGCGGCC GGGCTTgGG  
101 GTGTGAAGGT TTTGCGTTAT GAGATTAAAG ACTTGGTTCC GCCGCAAGAA  
151 ATCCTTCGCT CAATGCAGGC GCAAATTACT GCCGAACGCG AAAAACGCGC  
201 CCGTATCGCC GAATCCGAAG GTCGTAAAAT CGAACAAATC AACCTTGCCA  
251 GTGGTCAGCG CGAAGCCGAA ATCCAACAAT CCGAAGGCGA GGCTCAGGCT  
301 GCGGTCAATG CGTCAAATGC CGAGAAAATC GCCCGCATCA ACCGCGCCAA  
351 AGGTGAAGCG GAATCCTTGC GCCTTGTGTC CGAAGCCAAT GCCGAAGCCA  
401 TCCGTCAAAT TGCCGCCGCC CTTCAAACCC AAGGCGGTGC GGATGCGGTC  
451 AATCTGAAGA TTGCGGAACA ATACGTCGCT GCGTTCAACA ATCTTGCCAA  
501 AGAAAGCAAT ACGCTGATTA TGCCCGCCAA TGTTGCCGAC ATCGGCAGCC  
551 TGATTTCTGC CGGTATGAAA ATTATCGACA GCAGCAAAAC CGCCAAaTAA

This corresponds to the amino acid sequence <SEQ ID 9; ORF 519>:

m519.pep (partial)  
1 ..SVIGRMELDK TFEERDEINS TVVAALDEAA GAWGVKVLRY EIKDLVPPQE  
51 ILRSMQAQIT AEREKRARIA ESEGRKIEQI NLASGQREAE IQQSEGEAQA  
101 AVNASNAEKI ARINRAKGEA ESLRLVAEAN AEAIRQIAAA LQTQGGADAV  
151 NLKIAEQYVA AFNNLAKESN TLIMPANVAD IGSLISAGMK IIDSSKTAK\*

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 10>:

g519.seq  
1 atggaatttt tcattatctt gttggcagcc gtcgccgttt tcggcttcaa  
51 atcctttgtc gtcaccccc agcaggaagt ccacgttgtc gaaaggctcg

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101 ggcgtttcca tcgcgccttg acggccggtt tgaatatttt gattcccttt
151 atcgaccgcg tcgcctaccg ccattcgctg aaagaaatcc ctttagacgt
201 acccagccag gtctgcatca cgcgcgataa tacgcaattg actgttgacg
251 gcatcatcta tttccaagta accgatccca aactcgectc atacggttcg
301 agcaactaca ttatggcaat taccagctt gcccaaacga cgctgegtgc
351 cgttateggg cgtatggagt tggacaaaac gtttgaagaa cgcgacgaaa
401 tcaacagtac cgtcgtctcc gccctcgatg aagccgcccg ggcttggggg
451 gtgaaagtcc tccgttacga aatcaaggat ttggttccgc egcaagaaat
501 ccttcgcgca atgcaggcac aaattacegc cgaacgcgaa aaacgcgccc
551 gtattgccga atccgaaggc cgtaaaatcg aacaaatcaa ccttgccagt
601 ggtcagcgtg aagccgaaat ccaacaatcc gaaggcgagg cteaggctgc
651 ggtcaatgag tccaatgccc agaataatcg ccgcataaac cgcgcctaac
701 gegaagcggg atccctgcgc ettggttgcg aagccaatgc cgaagccaac
751 cgtcaaattg ccgcccctt tcaaacccaa agcggggcgg atgcggtcaa
801 tctgaagatt gcgggacaa acgttaccgc gttcaaaaat cttgccaaag
851 aagacaatac gcggattaag cccgccagg ttgccgaaat cgggaaccct
901 aattttcggc ggcatgaaaa attttcgcca gaagcaaaaa cggccaaata
951 a

```

This corresponds to the amino acid sequence <SEQ ID 11; ORF 519.ng>:

g519.pep

```

1  MEFFIILLAA VAVFGFKSFV VIPQQEVHV ERLGRFHRAL TAGLNILIPF
51  IDRVAYRHSL KEIPLDVPSQ VCITRDNTQL TVDGIIFYQV TDPKLASYGS
101 SNYIMAITQL AQTTLRSVIG RMELDKTFEE RDEINSTVVS ALDEAAGAWG
151 VKVLRYEIKD LVPPQEILRA MQAQITAERE KRARIAESEG RKIEQINLAS
201 GQREAEIQQS EGQAQAVNA SNAEKIARIN RAKGEAESLR LVAEANAEN
251 RQIAAALQTQ SGADAVNLKI AGQYVTAFFN LAKEDNTRIK PAKVAEIGNP
301 NFRRHEKFSP EAKTAK*

```

ORF 519 shows 87.5% identity over a 200 aa overlap with a predicted ORF (ORF 519.ng) from *N. gonorrhoeae*:

m519/g519

m519.pep					10	20	30
					SVIGRMELDKTFEERDEINSTVVAALDEAA		
g519							
	90	100	110	120	130	140	
m519.pep	40	50	60	70	80	90	
	GAWGVKVLRYEIKDLVPPQEILRSMQAQITAEREKRARIAESEG	GRKIEQINLASGQREAE					
g519							
	150	160	170	180	190	200	
m519.pep	100	110	120	130	140	150	
	IQQSEGEAQAAVNASNAEKIARINRAKGEAESLRLVAEANA	EAIRQIAAALQTQGGADAV					
g519							
	210	220	230	240	250	260	
m519.pep	160	170	180	190	200		
	NLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL-ISAGMKIIDSSKTAK						
g519							
	270	280	290	300	310		

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 12>:

a519.seq

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```

1  ATGGAATTTT TCATTATCTT GCTGGCAGCC GTCGTTGTTT TCGGCTTCAA
51  ATCCTTTGTT GTCATCCCAC AGCAGGAAGT CCACGTTGTC GAAAGGCTCG
101 GGCCTTTCCA TCGCGCCCTG ACGGCCGGTT TGAATATTTT GATTCCCTTT
151 ATCGACCGCG TCGCCTACCG CCATTTCGCTG AAAGAAATCC CTTTAGACGT
201 ACCCAGCCAG GTCTGCATCA CGCGCGACAA TACGCAGCTG ACTGTTGACG
251 GTATCATCTA TTTCCAAGTA ACCGACCCCA AACTCGCCTC ATACGGTTTCG
301 AGCAACTACA TTATGGCGAT TACCCAGCTT GCCCAAACGA CGCTGCGTTC
351 CGTTATCGGG CGTATGGAAT TGGACAAAAC GTTTGAAGAA CGCGACGAAA
401 TCAACAGCAC CGTCGTCTCC GCCCTCGATG AAGCCGCCGG AGCTTGGGGT
451 GTGAAGGTTT TCGGTTATGA GATTAAAGAC TTGGTTCCGC CGCAAGAAAT
501 CCTTCGCTCA ATGCAGGCGC AAATTACTGC TGAACGCGAA AAACGCGCCC
551 GTATCGCCGA ATCCGAAGGT CGTAAAATCG AACAAATCAA CCTTGCCAGT
601 GGTCAGCGCG AAGCCGAAAT CCAACAATCC GAAGGCGAGG CTCAGGCTGC
651 GGTCATGCG TCAAATGCCG AGAAAATCGC CCGCATCAAC CGCGCCAAAG
701 GTGAAGCGGA ATCCTTGCGC CTTGTTGCCG AAGCCAATGC CGAAGCCATC
751 CGTCAAATTG CCGCCGCCCT TCAAACCCAA GCGGTGCGG ATGCGGTCAA
801 TCTGAAGATT GCGGAACAAT ACGTCGCGC GTTCAACAAT CTTGCCAAAG
851 AAAGCAATAC GCTGATTATG CCCGCCAATG TTGCCGACAT CGGCAGCCTG
901 ATTTCTGCCG GTATGAAAAT TATCGACAGC AGCAAAACCG CCAAATAA

```

This corresponds to the amino acid sequence <SEQ ID 13; ORF 519.a>:

```

a519.pep
1  MEFFIILLAA VVVFGEKSFV VIPQEVHV V ERLGRFHRAL TAGLNILIPF
51  IDRVAIRHSL KEIPLDVPSQ VCITRDNTQL TVDGIYFQV TDPKLASYGS
101 SNYIMAITQL AQTTLRSVIG RMELDKTFEE RDEINSTVVS ALDEAAGAWG
151 VKVLRYEIKD LVPPQEILRS MQAQITAERE KRARIAESEGRKIEQINLAS
201 GQREAEIQQS EGEAQA AVNA SNAEKIARIN RAKGEAESLR LVAEANAEAI
251 RQIAAALQTQ GGADEVNLKI AEQYVAAFNN LAKESNTLIM PANVADIGSL
301 ISAGMKIIDS SKTAK*

m519/a519   ORFs 519 and 519.a showed a 99.5% identity in 199 aa overlap

m519.pep
10 20 30
SVIGRMELDKTFEERDEINSTVVAALDEAA
|||||:|||||
a519  YFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIGRMELDKTFEERDEINSTVVSALDEAA
90 100 110 120 130 140

m519.pep
40 50 60 70 80 90
GAWGVKVLRYEIKDLVPPQEILRSMAQITAEREKRARIAESEGRKIEQINLASGQREAE
|||||:|||||
a519  GAWGVKVLRYEIKDLVPPQEILRSMAQITAEREKRARIAESEGRKIEQINLASGQREAE
150 160 170 180 190 200

m519.pep
100 110 120 130 140 150
IQQSEGEAQA AVNASNAEKIARINRAKGEAESLRLVAEANAEAIRQIAAALQTQGGADAV
|||||:|||||
a519  IQQSEGEAQA AVNASNAEKIARINRAKGEAESLRLVAEANAEAIRQIAAALQTQGGADAV
210 220 230 240 250 260

m519.pep
160 170 180 190 200
NLKIAEQYVAAFNNLAKESNTLIMPANVADIGSLISAGMKIIDSSKTAKX
|||||:|||||
a519  NLKIAEQYVAAFNNLAKESNTLIMPANVADIGSLISAGMKIIDSSKTAKX
270 280 290 300 310

```

Further work revealed the following DNA sequence identified in *N. meningitidis* <SEQ ID 14>:

m519-1.seq



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```

1  ATGGAATTTT TCATTATCTT GTTGGTAGCC GTCGCCGTTT TCGGTTTCAA
51  ATCCTTTGTT GTCATCCAC AACAGGAAGT CCACGTTGTC GAAAGGCTGG
101 GCGTTTCCA TCGCGCCCTG ACGGCGGTT TGAATATTTT GATTCCCTTT
151 ATCGACCGCG TCGCCTACCG CCATTGCTG AAAGAAATCC CTTTAGACGT
201 ACCCAGCCAG GTCTGCATCA CGCGCGACAA TACGCAGCTG ACTGTTGACG
251 GCATCATCTA TTTCCAAGTA ACCGACCCCA AACTCGCCTC ATACGGTTTCG
301 AGCAACTACA TTATGGCGAT TACCCAGCTT GCCCAAACGA CGCTGCGTTC
351 CGTTATCGGG CGTATGGAGT TGGACAAAAC GTTTGAAGAA CGCGACGAAA
401 TCAACAGTAC TGTGTGTCG GCTTTGGACG AGGCGGCCGG GGCTTGGGGT
451 GTGAAGGTTT TGCATTATGA GATTAAAGAC TTGGTTCCGC CGCAAGAAAT
501 CCTTCGCTCA ATGCAGGCGC AAATTACTGC CGAACGCGAA AAACGCGCCC
551 GTATCGCCGA ATCCGAAGGT CGTAAAATCG AACAAATCAA CCTTGCCAGT
601 GGTCAAGCGC AAGCCGAAAT CCAACAATCC GAAGGCGAGG CTCAGGCTGC
651 GGTCAATGCG TCAAATGCCG AGAAAATCGC CCGCATCAAC CGCGCCAAAG
701 GTGAAGCGGA ATCCTTGCGC CTTGTTGCCG AAGCCAATGC CGAAGCCATC
751 CGTCAAATG CCGCGGCCCT TCAAACCCAA GGCGGTGCGG ATGCGGTCAA
801 TCTGAAGATT GCGGAACAAT ACGTCGCTGC GTTCAACAAT CTTGCCAAAG
851 AAAGCAATAC GCTGATTATG CCGCCAATG TTGCCGACAT CGGCAGCCTG
901 ATTTCTGCCG GTATGAAAAT TATCGACAGC AGCAAAACCG CCAAATAA

```

This corresponds to the amino acid sequence <SEQ ID 15; ORF 519-1>:

m519-1.

```

1  MEFFIILLVA VAVFGFKSFV VIPOQEVHVV ERLGRFHRAL TAGLNILIPF
51  IDRVAYRHSL KEIPLDVPSQ VCITRDNTQL TVDGIIYFQV TDPKLASYGS
101 SNYIMAITQL AQTTLRSVIG RMELDKTFEE RDEINSTVVA ALDEAAGAWG
151 VKVLYEIKD LVPPQEILRS MQAQITAERE KRARIAESEG RKIEQINLAS
201 GQREAEIQS EGEAQAAVNA SNAEKIARIN RAKGEAESLR LVAEANAEEI
251 RQIAAALQTQ GGADAVNLKI AEQYVAAFNN LAKESNTLIM PANVADIGSL
301 ISAGMKIIDS SKTAK*

```

The following DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 16>:

g519-1.seq

```

1  ATGGAATTTT TCATTATCTT GTTGGCAGCC GTCGCCGTTT TCGGCTTCAA
51  ATCCTTTGTC GTCATCCCCC AGCAGGAAGT CCACGTTGTC GAAAGGCTCG
101 GCGTTTCCA TCGCGCCCTG ACGGCGGTT TGAATATTTT GATTCCCTTT
151 ATCGACCGCG TCGCCTACCG CCATTGCTG AAAGAAATCC CTTTAGACGT
201 ACCCAGCCAG GTCTGCATCA CGCGCGATAA TACGCAATTG ACTGTTGACG
251 GCATCATCTA TTTCCAAGTA ACCGATCCCA AACTCGCCTC ATACGGTTTCG
301 AGCAACTACA TTATGGCAAT TACCCAGCTT GCCCAAACGA CGCTGCGTTC
351 CGTTATCGGG CGTATGGAGT TGGACAAAAC GTTTGAAGAA CGCGACGAAA
401 TCAACAGTAC CGTCGTCTCC GCCCTCGATG AAGCCGCCGG GGCTTGGGGT
451 GTGAAAGTCC TCCGTTACGA AATCAAGGAT TTGGTTCCGC CGCAAGAAAT
501 CCTTCGCGCA ATGCAGGCAC AAATTACCGC CGAACGCGAA AAACGCGCCC
551 GTATTGCCGA ATCCGAAGGC CGTAAAATCG AACAAATCAA CCTTGCCAGT
601 GGTCAAGCGT AAGCCGAAAT CCAACAATCC GAAGGCGAGG CTCAGGCTGC
651 GGTCAATGCG TCCAATGCCG AGAAAATCGC CCGCATCAAC CGCGCCAAAG
701 GCGAAGCGGA ATCCCTGCGC CTTGTTGCCG AAGCCAATGC CGAAGCCATC
751 CGTCAAATG CCGCGGCCCT TCAAACCCAA GGCGGGGCGG ATGCGGTCAA
801 TCTGAAGATT GCGGAACAAT ACGTAGCCGC GTTCAACAAT CTTGCCAAAG
851 AAAGCAATAC GCTGATTATG CCGCCAATG TTGCCGACAT CGGCAGCCTG
901 ATTTCTGCCG GCATGAAAAT TATCGACAGC AGCAAAACCG CCAAATAA

```

This corresponds to the amino acid sequence <SEQ ID 17; ORF 519-1.ng>:

g519-1.pep

```

1  MEFFIILLAA VAVFGFKSFV VIPOQEVHVV ERLGRFHRAL TAGLNILIPF
51  IDRVAYRHSL KEIPLDVPSQ VCITRDNTQL TVDGIIYFQV TDPKLASYGS
101 SNYIMAITQL AQTTLRSVIG RMELDKTFEE RDEINSTVVS ALDEAAGAWG
151 VKVLYEIKD LVPPQEILRA MQAQITAERE KRARIAESEG RKIEQINLAS
201 GQREAEIQS EGEAQAAVNA SNAEKIARIN RAKGEAESLR LVAEANAEEI
251 RQIAAALQTQ GGADAVNLKI AEQYVAAFNN LAKESNTLIM PANVADIGSL
301 ISAGMKIIDS SKTAK*

```

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m519-1/g519-1 ORFs 519-1 and 519-1.ng showed a 99.0% identity in 315 aa overlap

	10	20	30	40	50	60
g519-1.pep	MEFFIILLAAVAVFGFKSFVVI PQQEVHVVERLGRFHRALTAGLNILIPFIDRVAYRHSL					
	:					
m519-1	MEFFIILLVAVAVFGFKSFVVI PQQEVHVVERLGRFHRALTAGLNILIPFIDRVAYRHSL					
	10	20	30	40	50	60
	70	80	90	100	110	120
g519-1.pep	KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG					
	:					
m519-1	KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG					
	70	80	90	100	110	120
	130	140	150	160	170	180
g519-1.pep	RMELDKTFEERDEINSTVVSALDEAAGAWGVKVLRYEIKDLVPPQEILRAMQAQITAERE					
	:					
m519-1	RMELDKTFEERDEINSTVVAALDEAAGAWGVKVLRYEIKDLVPPQEILRSMAQITAERE					
	130	140	150	160	170	180
	190	200	210	220	230	240
g519-1.pep	KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR					
	:					
m519-1	KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR					
	190	200	210	220	230	240
	250	260	270	280	290	300
g519-1.pep	LVAEANAIAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL					
	:					
m519-1	LVAEANAIAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL					
	250	260	270	280	290	300
	310					
g519-1.pep	ISAGMKIIDSSKTAKX					
m519-1	ISAGMKIIDSSKTAKX					
	310					

The following DNA sequence was identified in *N. meningitidis* <SEQ ID 18>:

a519-1.seq

1	ATGGAATTTT	TCATTATCTT	GCTGGCAGCC	GTCGTTGTTT	TCGGCTTCAA
51	ATCCTTTGTT	GTCATCCAC	AGCAGGAAGT	CCACGTTGTC	GAAAGGCTCG
101	GGCGTTTCCA	TCGCGCCCTG	ACGGCCGGTT	TGAATATTTT	GATTCCTTTT
151	ATCGACCGCG	TCGCCTACCG	CCATTGCTG	AAAGAAATCC	CTTAGACGT
201	ACCCAGCCAG	GTCTGCATCA	CGCGCGACAA	TACGCAGCTG	ACTGTTGACG
251	GTATCATCTA	TTTCCAAGTA	ACCGACCCCA	AACTCGCCTC	ATACGGTTCTG
301	AGCAACTACA	TTATGGCGAT	TACCCAGCTT	GCCCAAACGA	CGCTGCGTTC
351	CGTTATCGGG	CGTATGGAAT	TGGACAAAAC	GTTTGAAGAA	CGCGACGAAA
401	TCAACAGCAC	CGTCGTCTCC	GCCCTCGATG	AAGCCGCCGG	AGCTTGGGGT
451	GTGAAGGTTT	TGCGTTATGA	GATTAAAGAC	TTGGTTCCGC	CGCAAGAAAT
501	CCTTCGCTCA	ATGCAGGCGC	AAATTACTGC	TGAACGCGAA	AAACGCGCCC
551	GTATCGCCGA	ATCCGAAGGT	CGTAAAATCG	AACAAATCAA	CCTTGCCAGT
601	GGTCAGCGCG	AAGCCGAAAT	CCAACAATCC	GAAGGCGAGG	CTCAGGCTGC
651	GGTCAATGCG	TCAAATGCCG	AGAAAATCGC	CCGCATCAAC	CGCGCCAAAG
701	GTGAAGCGGA	ATCCTTGCGC	CTTGTTGCCG	AAGCCAATGC	CGAAGCCATC
751	CGTCAAATTG	CCGCCGCCCT	TCAAACCCAA	GGCGGTGCGG	ATGCGGTCAA
801	TCTGAAGATT	GCGGAACAAT	ACGTGCGCCG	GTTCAACAAT	CTTGCCAAAG
851	AAAGCAATAC	GCTGATTATG	CCCGCCAATG	TTGCCGACAT	CGGCAGCCTG
901	ATTTCTGCCG	GTATGAAAAT	TATCGACAGC	AGCAAAACCG	CCAAATAA

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This corresponds to the amino acid sequence <SEQ ID 19; ORF 519-1.a>:

a519-1.pep.

```

1  MEFFIILLAA VVVFGEKSFV VIPQQEVHV ERLGRFHRAL TAGLNILIPF
51  IDRVAYRHSL KEIPLDVPSQ VCITRDNTQL TVDGIIYFQV TDPKLASYGS
101 SNYIMAITQL AQTTLRSVIG RMELDKTFEE RDEINSTVVS ALDEAAGAWG
151 VKVLRYEIKD LVPPQEILRS MQAQITAERE KRARIAESEG RKIEQINLAS
201 GQREAEIQQS EGEAQAANA SNAEKIARIN RAKGEAESLR LVAEANAIAI
251 RQIAAALQTQ GGADAVNLKI AEQYVAAFNN LAKESNTLIM PANVADIGSL
301 ISAGMKIIDS SKTAK*

```

m519-1/a519-1 ORFs 519-1 and 519-1.a showed a 99.0% identity in 315 aa overlap

a519-1.pep	10	20	30	40	50	60
	MEFFIILLAAVVVFGEKSFVIPQQEVHVVERLGRFHRALTAGLNILIPFIDRVAYRHSL					
m519-1	MEFFIILLVAVAVVFGEKSFVIPQQEVHVVERLGRFHRALTAGLNILIPFIDRVAYRHSL					
	10	20	30	40	50	60
a519-1.pep	70	80	90	100	110	120
	KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG					
m519-1	KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG					
	70	80	90	100	110	120
a519-1.pep	130	140	150	160	170	180
	RMELDKTFEERDEINSTVVSALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQQAITAERE					
m519-1	RMELDKTFEERDEINSTVVAALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQQAITAERE					
	130	140	150	160	170	180
a519-1.pep	190	200	210	220	230	240
	KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAANASNAEKIARINRAKGEAESLR					
m519-1	KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAANASNAEKIARINRAKGEAESLR					
	190	200	210	220	230	240
a519-1.pep	250	260	270	280	290	300
	LVAEANAIAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL					
m519-1	LVAEANAIAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL					
	250	260	270	280	290	300
a519-1.pep	310					
	ISAGMKIIDSSKTAKX					
m519-1	ISAGMKIIDSSKTAKX					
	310					

576 and 576-1 gnm22.seq

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 20>:

m576.seq.. (partial)

```

1  ..ATGCAGCAGG CAAGCTATGC GATGGGCGTG GACATCGGAC GCTCCCTGAA
51  GCAAATGAAG GAACAGGGCG CGGAAATCGA TTTGAAAGTC TTTACCGAAG
101 CCATGCAGGC AGTGTATGAC GGCAAAGAAA TCAAATGAC CGAAGAGCAG
151 GCTCAGGAAG TCATGATGAA ATTCCTTCAG GAACAACAGG CTAAAGCCGT
201 AGAAAAACAC AAGGCGGACG CGAAGGCCAA TAAAGAAAAA GGCGAAGCCT

```

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```

251 TTCTGAAAGA AAATGCCGCC AAAGACGGCG TGAAGACCAC TGCTTCCGGC
301 CTGCAATACA AAATCACCAA ACAGGGCGAA GGCAAACAGC CGACCAAAGA
351 CGACATCGTT ACCGTGGAAT ACGAAGGCCG CCTGATTGAC GGTACGGTAT
401 TCGACAGCAG CAAAGCCAAC GGCGGCCCGG TCACCTTCCC TTGAGCCAA
451 GTGATTCCGG GTTGGACCGA AGCGGTACAG CTTCTGAAAG AAGGCGGCGA
501 AGCCACGTTT TACATCCCGT CCAACCTTGC CTACCGCGAA CAGGGTGCGG
551 GCGACAAAAT CGGTCCGAAC GCCACTTTGG TATTTGATGT GAAACTGGTC
601 AAAATCGGCG CACCCGAAAA CGCGCCCGCC AAGCAGCCGG CTCAAGTCGA
651 CATCAAAAAA GTAAATTAA

```

This corresponds to the amino acid sequence <SEQ ID 21; ORF 576>:

```

m576.pep.. (partial)
1  ..MQQASYAMGV DIGRSLKQMK EQGAEIDLKV FTEAMQAVYD GKEIKMTEEQ
51  AQEVMKFLQ EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVKTTASG
101 LQYKITKQGE GKQPTKDDIV TVEYEGRLID GTVFDSSKAN GGPVTFPLSQ
151 VIPGWTEGVQ LLKEGGEATF YIPSNLAYRE QGAGDKIGPN ATLVFDVKLV
201 KIGAPENAPA KQPAQVDIKK VN*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 22>:

```

g576.seq.. (partial)
1  ..atgggctggt acatcgagcgt ctccctgaaa caaatgaagg aacagggcgc
51  ggaaatcgat ttgaaagtct ttaccgatgc catgcaggca gtgtatgacg
101 gcaaagaaat caaaatgacc gaagagcagg cccaggaagt gatgatgaaa
151 ttctgacagg agcagcaggc taaagccgta gaaaaacaca aggcggatgc
201 gaaggccaac aaagaaaaag gcgaagcctt cctgaaggaa aatgccgccg
251 aagacggcgt gaagaccact gcttccggtc tgcagtacaa aatcaccaaa
301 cagggtgaag gcaaacagcc gacaaaagac gacatcgta ccgtggaata
351 cgaaggccgc ctgattgacg gtaccgtatt cgacagcagc aaagccaacg
401 gcggcccgcc caccttccct ttgagccaag tgattccggg ttggaccgaa
451 ggcgtacggc ttctgaaaga aggcggcgaa gccacgttct acatcccgct
501 caaccttgcc taccgcgaac aggggtcggg cgaaaaaatc ggtccgaacg
551 ccactttggt atttgacgtg aaactggtca aaatcggcgc acccgaaaaa
601 gcgcccgcga agcagccgga tcaagtcgac atcaaaaaag taaattaa

```

This corresponds to the amino acid sequence <SEQ ID 23; ORF 576.ng>:

```

g576.pep.. (partial)
1  ..MGVDIGRSLK QMKEQGAIEID LKVFTDAMQA VYDGKEIKMT EEQAQEVMMK
51  FLQEQQAKAV EKHKADAKAN KEKGEAFLKE NAAEDGVKTT ASGLQYKITK
101 QGEGKQPTKD DIVTVEYEGR LIDGTVFDSS KANGGPATFP LSQVIPGWTE
151 GVRLLKEGGE ATFFYIPSNLA YREQGAGEKI GPNATLVFDV KLVKIGAPEN
201 APAKQPDQVD IKKVN*

```

Computer analysis of this amino acid sequence gave the following results:

Homology with a predicted ORF from *N. gonorrhoeae*

m576/g576 97.2% identity in 215 aa overlap

```

              10      20      30      40      50      60
m576.pep  MQQASYAMGV DIGRSLKQMK EQGAEIDLKV FTEAMQAVY DGKEIKMTEE QAQEVMMKFLQ
              |||
g576      MGVDIGRSLKQMK EQGAEIDLKV FTEAMQAVY DGKEIKMTEE QAQEVMMKFLQ
              10      20      30      40      50

              70      80      90      100     110     120
m576.pep  EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVKTTAS GLQYKITK QGEGKQPTKDDIV
              |||
g576      EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVKTTAS GLQYKITK QGEGKQPTKDDIV
              60      70      80      90      100     110

```

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	130	140	150	160	170	180
m576.pep	TVEYEGRLIDGTVFDSSKANGGPVTFPLSQVIPGWTEGVQLLKEGGEATFYIPSNLAYRE					
g576	TVEYEGRLIDGTVFDSSKANGGPATFPLSQVIPGWTEGVRLLEKGEATFYIPSNLAYRE					
	120	130	140	150	160	170
	190	200	210	220		
m576.pep	QGAGDKIGPNATLVFDVKLVKIGAPENAPAKQPAQVDIKKVN					
g576	QGAGEKIGPNATLVFDVKLVKIGAPENAPAKQPDQVDIKKVN					
	180	190	200	210		

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 24>:

```
a576.seq
1   ATGAACACCA TTTTCAAAT CAGCGCACTG ACCCTTTCCG CCGCTTTGGC
51  ACTTTCCGCC TGCGGCAAAA AAGAAGCCGC CCCC GCATCT GCATCCGAAC
101 CTGCCGCCGC TTCTTCCGCG CAGGGCGACA CCTCTTCGAT CGGCAGCAGC
151 ATGCAGCAGG CAAGCTATGC GATGGGCGTG GACATCGGAC GCTCCCTGAA
201 GCAAATGAAG GAACAGGGCG CGGAAATCGA TTTGAAAGTC TTTACCGAAG
251 CCATGCAGGC AGTGTATGAC GGCAAAGAAA TCAAATGAC CGAAGAGCAG
301 GCTCAGGAAG TCATGATGAA ATTCCTTCAG GAACAACAGG CTAAAGCCGT
351 AGAAAAACAC AAGGCGGACG CGAAGGCCAA TAAAGAAAAA GGCGAAGCCT
401 TTCTGAAAGA AAATGCCGCC AAAGACGGCG TGAAGACCAC TGCTTCCGGC
451 CTGCAATACA AAATCACCAA ACAGGGCGAA GGCAAACAGC CGACCAAAGA
501 CGACATCGTT ACCGTGGAAT ACGAAGGCCG CCTGATTGAC GGTACGGTAT
551 TCGACAGCAG CAAAGCCAAC GCGCGCCCGG TCACCTTCCC TTTGAGCCAA
601 GTGATTCTGG GTTGGACCGA AGGCGTACAG CTTCTGAAAG AAGGCGGCCA
651 AGCCACGTTT TACATCCCGT CCAACCTTGC CTACCGCGAA CAGGGTGCGG
701 GCGACAAAAT CGGCCCGAAC GCCACTTTGG TATTTGATGT GAAACTGGTC
751 AAAATCGGCG CACCCGAAAA CGCGCCCGCC AAGCAGCCGG CTCAAGTCGA
801 CATCAAAAAA GTAAATTAA
```

This corresponds to the amino acid sequence <SEQ ID 25; ORF 576.a>:

```
a576.pep
1   MNTIFKISAL TLSAALLSA CGKKEAAPAS ASEPAAASSA QGDTSSIGST
51  MQQASYAMGV DIGRSLKQMK EQGAEIDLKV FTEAMQAVYD GKEIKMTEEQ
101 AQEVMKFLQ EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVKTTASG
151 LQYKITQGE GKQPTKDDIV TVEYEGRLID GTVFDSSKAN GGPVTFPLSQ
201 VILGWTEGVQ LLKEGGEATF YIPSNLAYRE QGAGDKIGPN ATLVFDVKLV
251 KIGAPENAPA KQPAQVDIKK VN*
```

m576/a576      ORFs 576 and 576.a showed a 99.5% identity in 222 aa overlap

		10	20	30		
m576.pep		MQQASYAMGV DIGRSLKQMK EQGAEIDLKV				
a576		CGKKEAAPAS ASEPA <del>AA</del> SSA QGDTSSIGSTM MQQASYAMGV DIGRSLKQMK EQGAEIDLKV				
	30	40	50	60	70	80
	40	50	60	70	80	90
m576.pep	FTEAMQAVYD GKEIKMTEEQAQ EVMKFLQEQQAKAVEKHKADAKANKEKGEAFLENAA					
a576	FTEAMQAVYD GKEIKMTEEQAQ EVMKFLQEQQAKAVEKHKADAKANKEKGEAFLENAA					
	90	100	110	120	130	140
	100	110	120	130	140	150
m576.pep	KDGVKTTASGLQYKITKQGE GKQPTKDDIV TVEYEGRLIDGTVFDSSKANGGPVTFPLSQ					
a576	KDGVKTTASGLQYKITKQGE GKQPTKDDIV TVEYEGRLIDGTVFDSSKANGGPVTFPLSQ					
	150	160	170	180	190	200

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	160	170	180	190	200	210
m576.pep	VIPGWTEGVQLLKEGGEATFYIPSNLAYREQGAGDKIGPNATLVFDVKLVKIGAPENAPA					
a576	VILGWTEGVQLLKEGGEATFYIPSNLAYREQGAGDKIGPNATLVFDVKLVKIGAPENAPA					
	210	220	230	240	250	260
	220					
m576.pep	KQPAQVDIKKVN					
a576	KQPAQVDIKKVN					
	270					

Further work revealed the following DNA sequence identified in *N. meningitidis* <SEQ ID 26>:

m576-1.seq

1	ATGAACACCA	TTTTCAAAAT	CAGCGCACTG	ACCCTTTCCG	CCGCTTTGGC
51	ACTTTCCGCC	TGCGGCAAAA	AAGAAGCCGC	CCCCGCATCT	GCATCCGAAC
101	CTGCCGCCGC	TTCTTCGCG	CAGGGCGACA	CCTCTTCGAT	CGGCAGCACG
151	ATGCAGCAGG	CAAGCTATGC	GATGGGCGTG	GACATCGGAC	GCTCCCTGAA
201	GCAAATGAAG	GAACAGGGCG	CGGAAATCGA	TTTGAAAGTC	TTTACCGAAG
251	CCATGCAGGC	AGTGTATGAC	GGCAAAGAAA	TCAAAATGAC	CGAAGAGCAG
301	GCTCAGGAAG	TCATGATGAA	ATTCCTTCAG	GAACAACAGG	CTAAAGCCGT
351	AGAAAAACAC	AAGGCGGACG	CGAAGGCCAA	TAAAGAAAAA	GGCGAAGCCT
401	TTCTGAAAGA	AAATGCCGCC	AAAGACGGCG	TGAAGACCAC	TGCTTCCGGC
451	CTGCAATACA	AAATCACCAA	ACAGGGCGAA	GGCAAACAGC	CGACCAAAGA
501	CGACATCGTT	ACCGTGGAAT	ACGAAGGCCG	CCTGATTGAC	GGTACCGTAT
551	TCGACAGCAG	CAAAGCCAAC	GGCGGCCCGG	TCACCTTCCC	TTTGAGCCAA
601	GTGATTCCGG	GTTGGACCGA	AGGCGTACAG	CTTCTGAAAG	AAGGCGGCGA
651	AGCCACGTTT	TACATCCCGT	CCAACCTTGC	CTACCGCGAA	CAGGGTGCGG
701	GCGACAAAAT	CGGTCCGAAC	GCCACTTTGG	TATTTGATGT	GAAACTGGTC
751	AAAATCGGCG	CACCCGAAAA	CGCGCCCGCC	AAGCAGCCGG	CTCAAGTCGA
801	CATCAAAAAA	GTAATTAA			

This corresponds to the amino acid sequence <SEQ ID 27; ORF 576-1>:

m576-1.pep

1	MNTIFKISAL	TLAALALSA	CGKKEAPAS	ASEPAAASSA	QGDTSISIGT
51	MQQASYAMGV	DIGRSLKQMK	EQGAEIDLKV	FTEAMQAVYD	GKEIKMTEEQ
101	AQEVMMKFLQ	EQQAKAVEKH	KADAKANKEK	GEAFLKENAA	KDGVKTASG
151	LQYKITKQGE	GKQPTKDDIV	TVEYEGRLID	GTVFDSSKAN	GGPVTFFLSQ
201	VIPGWTEGVQ	LLKEGGEATF	YIPSNLAYRE	QGAGDKIGPN	ATLVFDVKLV
251	KIGAPENAPA	KQPAQVDIKK	VN*		

The following DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 28>:

g576-1.seq

1	ATGAACACCA	TTTTCAAAAT	CAGCGCACTG	ACCCTTTCCG	CCGCTTTGGC
51	ACTTTCCGCC	TGCGGCAAAA	AAGAAGCCGC	CCCCGCATCT	GCATCCGAAC
101	CTGCCGCCGC	TTCTGCGCG	CAGGGCGACA	CCTCTTCAAT	CGGCAGCACG
151	ATGCAGCAGG	CAAGCTATGC	AATGGGCGTG	GACATCGGAC	GCTCCCTGAA
201	ACAAATGAAG	GAACAGGGCG	CGGAAATCGA	TTTGAAAGTC	TTTACCGATG
251	CCATGCAGGC	AGTGTATGAC	GGCAAAGAAA	TCAAAATGAC	CGAAGAGCAG
301	CCCCAGGAAG	TGATGATGAA	ATTCCTGCAG	GAGCAGCAGG	CTAAAGCCGT
351	AGAAAAACAC	AAGGCGGATG	CGAAGGCCAA	CAAAGAAAAA	GGCGAAGCCT
401	TCCTGAAGGA	AAATGCCGCC	AAAGACGGCG	TGAAGACCAC	TGCTTCCGGT
451	CTGCAGTACA	AAATCACCAA	ACAGGGTGAA	GGCAAACAGC	CGACAAAAGA
501	CGACATCGTT	ACCGTGGAAT	ACGAAGGCCG	CCTGATTGAC	GGTACCGTAT
551	TCGACAGCAG	CAAAGCCAAC	GGCGGCCCGG	CCACCTTCCC	TTTGAGCCAA
601	GTGATTCCGG	GTTGGACCGA	AGGCGTACGG	CTTCTGAAAG	AAGGCGGCGA
651	AGCCACGTTT	TACATCCCGT	CCAACCTTGC	CTACCGCGAA	CAGGGTGCGG
701	GCGAAAAAAT	OGGTCCGAAC	GCCACTTTGG	TATTTGACGT	GAAACTGGTC
751	AAAATCGGCG	CACCCGAAAA	CGCGCCCGCC	AAGCAGCCGG	ATCAAGTCGA

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801 CATCAAAAAA GTAAATTAA

This corresponds to the amino acid sequence &lt;SEQ ID 29; ORF 576-1.ng&gt;:

g576-1.pep

```

1  MNTIFKISAL TLSAALALSA CGKKEAAPAS ASEPAASAA QGDTSSIGST
51  MQQASYAMGV DIGRSLKQMK EQGAEIDLKV FTDAMQAVYD GKEIKMTEEQ
101 AQEVMKFLQ EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVKTTASG
151 LQYKITKQGE GKQPTKDDIV TVEYEGRLID GTVFDSSKAN GGPATFPLSQ
201 VIPGWTEGVR LLKEGGEATF YIPSNLAYRE QGAGEKIGPN ATLVFDVKLV
251 KIGAPENAPA KQPDQVDIKK VN*

```

g576-1/m576-1 ORFs 576-1 and 576-1.ng showed a 97.8% identity in 272 aa overlap

	10	20	30	40	50	60
g576-1.pep	MNTIFKISAL	TLSAALALSA	CGKKEAAPAS	ASEPAASAA	QGDTSSIGST	MQQASYAMGV
m576-1	MNTIFKISAL	TLSAALALSA	CGKKEAAPAS	ASEPAASSA	QGDTSSIGST	MQQASYAMGV
	10	20	30	40	50	60
	70	80	90	100	110	120
g576-1.pep	DIGRSLKQMK	EQGAEIDLKV	FTDAMQAVYD	GKEIKMTEEQ	AQEVMKFLQ	EQQAKAVEKH
m576-1	DIGRSLKQMK	EQGAEIDLKV	FTEAMQAVYD	GKEIKMTEEQ	AQEVMKFLQ	EQQAKAVEKH
	70	80	90	100	110	120
	130	140	150	160	170	180
g576-1.pep	KADAKANKEK	GEAFLENAA	AKDGVKTTAS	GLOQYKITKQ	GEGKQPTKDD	IVTVEYEGRLID
m576-1	KADAKANKEK	GEAFLENAA	AKDGVKTTAS	GLOQYKITKQ	GEGKQPTKDD	IVTVEYEGRLID
	130	140	150	160	170	180
	190	200	210	220	230	240
g576-1.pep	GTVFDSSKAN	GGPATFPLSQ	VIPGWTEGVR	LLKEGGEATF	YIPSNLAYRE	QGAGEKIGPN
m576-1	GTVFDSSKAN	GGPVTFPLSQ	VIPGWTEGV	QLKEGGEATF	YIPSNLAYRE	QGAGDKIGPN
	190	200	210	220	230	240
	250	260	270			
g576-1.pep	ATLVFDVKLV	KIGAPENAPA	KQPDQVDIKK	VN		
m576-1	ATLVFDVKLV	KIGAPENAPA	KQPAQVDIKK	VN		
	250	260	270			

The following DNA sequence was identified in *N. meningitidis* <SEQ ID 30>:

a576-1.seq

```

1  ATGAACACCA TTTTCAAAT CAGCGCACTG ACCCTTTCGG CCGCTTTGGC
51  ACTTTCGCCG TGCGGCAAAA AAGAAGCCGC CCCCGCATCT GCATCCGAAC
101 CTGCCGCCGC TTCTTCCGCG CAGGGCGACA CCTCTTCGAT CGGCAGCAGC
151 ATGCAGCAGG CAAGCTATGC GATGGGCGTG GACATCGGAC GCTCCCTGAA
201 GCAAATGAAG GAACAGGGCG CGGAAATCGA TTTGAAAGTC TTTACCGAAG
251 CCATGCAGGC AGTGTATGAC GGCAAAGAAA TCAAATGAC CGAAGAGCAG
301 GCTCAGGAAG TCATGATGAA ATTCCTTCAG GAACAACAGG CTAAGCCGT
351 AGAAAAACAC AAGGCGGACG CGAAGGCCAA TAAAGAAAAA GGCGAAGCCT
401 TTCTGAAAGA AAATGCCGCC AAAGACGGCG TGAAGACCAC TGCTTCCGGC
451 CTGCAATACA AAATCACCAA ACAGGGCGAA GGCAAACAGC CGACCAAAGA
501 CGACATCGTT ACCGTGGAAT ACGAAGGCCG CCTGATTGAC GGTACGGTAT
551 TCGACAGCAG CAAAGCCAAC GGCGGCCCGG TCACCTTCCC TTTGAGCCAA
601 TGATTCTGG GTTGGACCGA AGGCGTACAG CTCTGAAAG AAGGCGGCGA
651 AGCCACGTTC TACATCCCGT CCAACCTTGC CTACCGCGAA CAGGGTGCGG
701 GCGACAAAAT CGGCCCGAAC GCCACTTTGG TATTTGATGT GAAACTGGTC

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751 AAAATCGGCG CACCCGAAAA CGCGCCCGCC AAGCAGCCGG CTCAAGTCGA  
801 CATCAAAAAA GTAAATTAA

This corresponds to the amino acid sequence <SEQ ID 31; ORF 576-1.a>:

a576-1.pep

1 MNTIFKISAL TLSAALALSA CGKKEAAPAS ASEPAASSA QGDTSSIGST  
51 MQQASYAMGV DIGRSLKQMK EQGAEIDLKV FTEAMQAVYD GKEIKMTEEQ  
101 AQEVMMKFLQ EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVKTTASG  
151 LQYKITKQGE GKQPTKDDIV TVEYEGRLID GTVFDSSKAN GGPVTFPLSQ  
201 VILGWTEGVQ LLKEGGEATF YIPSNLAYRE QGAGDKIGPN ATLVFDVKLV  
251 KIGAPENAPA KQPAQVDIKK VN\*

a576-1/m576-1 ORFs 576-1 and 576-1.a 99.6% identity in 272 aa overlap

	10	20	30	40	50	60
a576-1.pep	MNTIFKISAL	TLSAALALS	ACGKKEAPAS	ASEPAASSA	QGDTSSIGST	MQQASYAMGV
m576-1	MNTIFKISAL	TLSAALALS	ACGKKEAPAS	ASEPAASSA	QGDTSSIGST	MQQASYAMGV
	10	20	30	40	50	60
	70	80	90	100	110	120
a576-1.pep	DIGRSLKQMK	EQGAEIDLKV	FTEAMQAVYD	GKEIKMTEE	QAQEVMMKFL	QEQAKAVEKH
m576-1	DIGRSLKQMK	EQGAEIDLKV	FTEAMQAVYD	GKEIKMTEE	QAQEVMMKFL	QEQAKAVEKH
	70	80	90	100	110	120
	130	140	150	160	170	180
a576-1.pep	KADAKANKEK	GEAFLENAA	KDGVKTTAS	GLQYKITKQ	GEGKQPTKDD	IVTVEYEGRLID
m576-1	KADAKANKEK	GEAFLENAA	KDGVKTTAS	GLQYKITKQ	GEGKQPTKDD	IVTVEYEGRLID
	130	140	150	160	170	180
	190	200	210	220	230	240
a576-1.pep	GTVFDSSKAN	GGPVTFPLSQ	VILGWTEGV	QLLKEGGEAT	FYIPSNLAYRE	QGAGDKIGPN
m576-1	GTVFDSSKAN	GGPVTFPLSQ	VILGWTEGV	QLLKEGGEAT	FYIPSNLAYRE	QGAGDKIGPN
	190	200	210	220	230	240
	250	260	270			
a576-1.pep	ATLVFDVKLV	KIGAPENAPA	KQPAQVDIKK	VNX		
m576-1	ATLVFDVKLV	KIGAPENAPA	KQPAQVDIKK	VNX		
	250	260	270			

919 and 919-2

gnm43.seq

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 32>:

m919.seq

1 ATGAAAAAAT ACCTATTCGG CGCCGCCCTG TACGGCATCG CCGCCGCCAT  
51 CCTCGCCGCC TGCCAAAGCA AGAGCATCCA AACCTTTCGG CAACCCGACA  
101 CATCCGTCAT CAACGGCCCG GACCGGCCGG TCGGCATCCC CGACCCCGCC  
151 GGAACGACGG TCGGCGGCGG CGGGGCCGTC TATACCGTTG TACCGCACCT  
201 GTCCCTGCCC CACTGGGCGG CGCAGGATTT CGCCAAAAGC CTGCAATCCT  
251 TCCGCCTCGG CTGCGCCAAT TTGAAAAACC GCCAAGGCTG GCAGGATGTG  
301 TGCGCCCAAG CCTTCAAAC CCCCCTCCAT TCCTTTCAGG CAAAACAGTT  
351 TTTTGAACGC TATTTACGC CGTGGCAGGT TGCAGGCAAC GGAAGCCTTG



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401 CCGGTACGGT TACCGGCTAT TACGAACCGG TGCTGAAGGG CGACGACAGG
451 CGGACGGCAC AAGCCCGCTT CCCGATTTAC GGTATTCCCG ACGATTTTAT
501 CTCCGTCCCC CTGCCTGCCG GTTTGCGGAG CGGAAAAGCC CTTGTCCGCA
551 TCAGGCAGAC GGGAAAAAAC AGCGGCACAA TCGACAATAC CGGCGGCACA
601 CATACCGCCG ACCTCTCCCG ATTCCCCATC ACCGCGCGCA CAACAGCAAT
651 CAAAGGCAGG TTTGAAGGAA GCCGCTTCCT CCCCTACCAC ACGCGCAACC
701 AAATCAACGG CGGCGCGCTT GACGGCAAAG CCCCATACT CCGTTACGCC
751 GAAGACCCTG TCGAACTTTT TTTTATGCAC ATCCAAGGCT CGGGCCGTCT
801 GAAAACCCCG TCCGGCAAAT ACATCCGCAT CGGCTATGCC GACAAAAACG
851 AACATCCyTA CGTTTCCATC GGACGCTATA TGGCGGATAA GGGCTACCTC
901 AAATCGGAC AAACCTCCAT GCAGGGCATT AAGTCTTATA TGCGGCAAAA
951 TCCGCAACGC CTCGCCGAAG TTTTGGGTCA AAACCCAGC TATATCTTTT
1001 TCCGCGAGCT TGCCGGAAGC AGCAATGACG GCCCTGTCGG CGCACTGGGC
1051 ACGCCGCTGA TGGGGGAATA TGCCGGCGCA GTCGACCGGC ACTACATTAC
1101 CTTGGGTGCG CCCTTATTG TCGCCACCGC CCATCCGGTT ACCCGCAAAG
1151 CCCTCAACCG CCTGATTATG GCGCAGGATA CCGGCAGCGC GATTAAAGGC
1201 GCGGTGCGCG TGGATTATTT TTGGGGATAC GGCGACGAAG CCGGCGAACT
1251 TGCCGGCAA CAGAAAACCA CGGGATATGT CTGGCAGCTC CTACCCAACG
1301 GTATGAAGCC CGAATACCGC CCGTAA

```

This corresponds to the amino acid sequence <SEQ ID 33; ORF 919>:

m919.pep

```

1 MKKYLFRAL YGIAAAILAA CQSKSIQTFP QPDTSVINGP DRPVGIPDPA
51 GTTVGGGAV YTVVPHLSLP HWAAQDFAKS LQSFRLGCAN LKNRQGWQDV
101 CAQAFQTPVH SFQAKQFFER YFTPWQVAGN GSLAGTVTGY YEPVLKGDDR
151 RTAQRFPFIY GIPDDFISVP LPAGLRSGKA LVRIQTGKN SGTIDNTGTT
201 HTADLSRFPI TARTTAIKGR FEGRFLPYH TRNQINGGAL DGKAPILGYA
251 EDPVELFFMH IQGSGRLKTP SGKYIRIGYA DKNEHPYVSI GRYMADKGYL
301 KLGQTSMQGI KSYMQRNPQR LAEVLGQNPS YIFFRELAGS SNDGPVGALG
351 TPLMGEYAGA VDRHYITLGA PLFVATAHPV TRKALNRLIM AQDTGSAIKG
401 AVRVDYFWGY GDEAGELAGK QKTTGYVWQL LPNGMKPEYR P*

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The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 34>:

m919-2.seq

```

1 ATGAAAAAAT ACCTATTCCG CGCCGCCCTG TACGGCATCG CCGCCGCCAT
51 CCTCGCGGCC TGCCAAAGCA AGAGCATCCA AACCTTTCCG CAACCCGACA
101 CATCCGTCAT CAACGGCCCG GACCGGCCGG TCGGCATCCC CGACCCCGCC
151 GGAACGACGG TCGGCGCGCG CGGGGCCGTC TATACCGTTG TACCGCACCT
201 GTCCCTGCCC CACTGGGCGG CGCAGGATTT CGCCAAAAGC CTGCAATCCT
251 TCCGCCTCGG CTGCGCCAAT TTGAAAAACC GCCAAGGCTG GCAGGATGTG
301 TGCGCCCAAG CCTTTC AAC CCCCCTCCAT TCCTTTCAGG CAAAACAGTT
351 TTTTGAACGC TATTTACGC CGTGGCAGGT TGCAGGCAAC GGAAGCCTTG
401 CCGGTACGGT TACCGGCTAT TACGAACCGG TGCTGAAGGG CGACGACAGG
451 CGGACGGCAC AAGCCCGCTT CCCGATTTAC GGTATTCCCG ACGATTTTAT
501 CTCCGTCCCC CTGCCTGCCG GTTTGCGGAG CGGAAAAGCC CTTGTCCGCA
551 TCAGGCAGAC GGGAAAAAAC AGCGGCACAA TCGACAATAC CGGCGGCACA
601 CATACCGCCG ACCTCTCCCG ATTCCCCATC ACCGCGCGCA CAACAGCAAT
651 CAAAGGCAGG TTTGAAGGAA GCCGCTTCCT CCCCTACCAC ACGCGCAACC
701 AAATCAACGG CGGCGCGCTT GACGGCAAAG CCCCATACT CCGTTACGCC
751 GAAGACCCTG TCGAACTTTT TTTTATGCAC ATCCAAGGCT CGGGCCGTCT
801 GAAAACCCCG TCCGGCAAAT ACATCCGCAT CGGCTATGCC GACAAAAACG
851 AACATCCCTA CGTTTCCATC GGACGCTATA TGGCGGATAA GGGCTACCTC
901 AAATCGGAC AAACCTCCAT GCAGGGCATT AAGTCTTATA TGCGGCAAAA
951 TCCGCAACGC CTCGCCGAAG TTTTGGGTCA AAACCCAGC TATATCTTTT
1001 TCCGCGAGCT TGCCGGAAGC AGCAATGACG GCCCTGTCGG CGCACTGGGC
1051 ACGCCGCTGA TGGGGGAATA TGCCGGCGCA GTCGACCGGC ACTACATTAC
1101 CTTGGGTGCG CCCTTATTG TCGCCACCGC CCATCCGGTT ACCCGCAAAG
1151 CCCTCAACCG CCTGATTATG GCGCAGGATA CCGGCAGCGC GATTAAAGGC

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1201 GCGGTGCGCG TGGATTATTT TTGGGGATAC GGCGACGAAG CCGGCCAACT  
 1251 TGCCGGCAAA CAGAAAACCA CGGGATATGT CTGGCAGCTC CTACCCAACG  
 1301 GTATGAAGCC CGAATACCGC CCGTAA

This corresponds to the amino acid sequence <SEQ ID 35; ORF 919-2>:

m919-2.pep

1 MKKYLFRAL YGIAAAILAA CQSKSIQTFP QPDTSVINGP DRPVGIPDPA  
 51 GTTVGGGGAV YTVVPHLSLP HWAAQDFAKS LQSFRLGCAN LKNRQGWQDV  
 101 CAQAFQTPVH SFQAKQFFER YFTPWQVAGN GSLAGTVTGY YEPVLKGDDR  
 151 RTAQAARFPIY GIPDDFISVP LPAGLRSGKA LVRIRQTGKN SGTIDNTGGT  
 201 HTADLSRFPI TARTTAIKGR FEGRFLPYH TRNQINGGAL DGKAPILGYA  
 251 EDPVELFFMH IQGSGRLKTP SGKYIRIGYA DKNEHPYVSI GRYMADKGYL  
 301 KLGQTSMQGI KSYMQRNPQR LAEVLGQNPS YIFFRELAGS SNDGPVAGLG  
 351 TPLMGEYAGA VDRHYITLGA PLFVATAHPV TRKALNRLIM AQDTGSAIKG  
 401 AVRVDYFWGY GDEAGELAGK QKTTGYVWQL LPNGMKPEYR P\*

The following partial DNA sequence was identified in *N.gonorrhoeae* <SEQ ID 36>:

g919.seq

1 ATGAAAAAAC ACCTGCTCCG CTCCGCCCTG TACGGcatCG CCGCCgccAT  
 51 CctcgCGGCC TGCCAAAgca gGAGCATCCA AACCTTTCCG CAACCCGACA  
 101 CATCCGTCAT CAACGGCCCG GACCGGCCCG CCGGCATCCC CGACCCCGCC  
 151 GGAACGACGG TTGCCGGCGG CGGGGCCGTC TATACCGTTG TGCCGACCT  
 201 GTCCATGCCC CACTGGGCGG CGCaggATTT TGCCAAAAGC CTGCAATCCT  
 251 TCCGCCTCGG CTGCGCCAAT TTGAAAAACC GCCAAGGCTG GCAGGATGTG  
 301 TGCGCCCAAG CCTTTCAAAC CCCCGTGCAT TCCTTTCAGG CAAAGcGgTT  
 351 TTTTGAACGC TATTTACGC cgtGGCaggt tgcaggcaAC GGAAGcCTTG  
 401 Caggtacggt TACCGGCTAT TACGAACCGG TGCTGAAGGG CGACGGCAGG  
 451 CGGACGGAAC GGCCCGCCTT CCCGATTAC GGTATTCCCG ACGATTTTAT  
 501 CTCCGTCCCG CTGCCTGCCG GTTTGCGGGG CGGAAAAAAC CTTGTCCGCA  
 551 TCAGGCAGac ggGAAAAAAC AGCGGCACGA TCGACAATGC CGGCGGCACG  
 601 CATACCGCCG ACCTCTCCCG ATTCCCATC ACCGCGCGCA CAACGGcaat  
 651 caaaGGCAGG TTTGAaggAA GCCGCTTCCT CCCTTACCAC ACGCGCAACC  
 701 AAAtcaacGG CGGcgcgCTT GACGGCAAag cccCATCCT CggttacgcC  
 751 GAagaccCcG tcgaacttTT TTTCATGCAC AtccaaggCT CGGGCCCGCT  
 801 GAAAACCCcg tccggcaaat acatCCGCAc cgggTacgcc gacAAAAACG  
 851 AACAtccgTa tgtttccatc ggACGctata TGGCGGACAA AGGCTACCTC  
 901 AAGctcgggc agACCTCGAT GCAGGgcatc aaagcCTATA TGCGGCAAAA  
 951 TCCGCAACGC CTCGCCAAG TTTTGGGTCA AAACCCAGC TATATCTTTT  
 1001 TCCGCGAGCT TGCCGGAAGC GGCAATGAGG GCCCGTCCG CGCACTGGGC  
 1051 ACGCCACTGA TGGGGGAATA CGCCGGCGCA ATCGACCGGC ACTACATTAC  
 1101 CTTGGGCGCG CCCTTATTG TCGCCACCGC CCATCCGGTT ACCCGCAAAG  
 1151 CCCTCAACCG CCTGATTATG GCGCAGGATA CAGGCAGCGC GATCAAAGGC  
 1201 GCGGTGCGCG TGGATTATTT TTGGGGTTAC GGCGACGAAG CCGGCGAACT  
 1251 TGCCGGCAAA CAGAAAACCA CGGGATACGT CTGGCAGCTC CTGCCCAACG  
 1301 GCATGAAGCC CGAATACCGC CCGTGA

This corresponds to the amino acid sequence <SEQ ID 37; ORF 919.ng>:

g919.pep

1 MKKHLLRSAL YGIAAAILAA CQSRSIQTFP QPDTSVINGP DRPAGIPDPA  
 51 GTTVAGGGAV YTVVPHLSMP HWAAQDFAKS LQSFRLGCAN LKNRQGWQDV  
 101 CAQAFQTPVH SFQAKRFFER YFTPWQVAGN GSLAGTVTGY YEPVLKGDGR  
 151 RTERARFPIY GIPDDFISVP LPAGLRGGKN LVRIRQTGKN SGTIDNAGGT  
 201 HTADLSRFPI TARTTAIKGR FEGRFLPYH TRNQINGGAL DGKAPILGYA  
 251 EDPVELFFMH IQGSGRLKTP SGKYIRIGYA DKNEHPYVSI GRYMADKGYL  
 301 KLGQTSMQGI KAYMRNPQR LAEVLGQNPS YIFFRELAGS GNEGPVAGLG  
 351 TPLMGEYAGA IDRHYITLGA PLFVATAHPV TRKALNRLIM AQDTGSAIKG  
 401 AVRVDYFWGY GDEAGELAGK QKTTGYVWQL LPNGMKPEYR P\*

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ORF 919 shows 95.9 % identity over a 441 aa overlap with a predicted ORF (ORF 919.ng) from *N. gonorrhoeae*:

m919/g919

m919.pep	10	20	30	40	50	60
	MKKYLFRALYGIAAAILAACQSKSIQTFFQPDTSVINGPDRPVGIPDPAGTTVGGGGAV					
g919	10	20	30	40	50	60
	MKKHLRLSALYGIAAAILAACQSKSIQTFFQPDTSVINGPDRPAGIPDPAGTTVAGGGAV					
m919.pep	70	80	90	100	110	120
	YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER					
g919	70	80	90	100	110	120
	YTVVPHLSMPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKRFFER					
m919.pep	130	140	150	160	170	180
	YFTPWQVAGNGSLAGTVTGYEPPVLKGDGDRRTAQARFPIYGIIPDDFISVPLPAGLRSGKA					
g919	130	140	150	160	170	180
	YFTPWQVAGNGSLAGTVTGYEPPVLKGDGRRTERARFPIYGIIPDDFISVPLPAGLRGKKN					
m919.pep	190	200	210	220	230	240
	LVRIRQTGKNSGTIDNTGGTHTADLSRFPI TARTTAIKGRFEGSRFLPYHTRNQINGGAL					
g919	190	200	210	220	230	240
	LVRIRQTGKNSGTIDNAGGHTADLSRFPI TARTTAIKGRFEGSRFLPYHTRNQINGGAL					
m919.pep	250	260	270	280	290	300
	DGKAPILGYAEDPVELFFMHIIQSGRLKTPSGKYIRIGYADKNEHPYVSI GRYMADKGYL					
g919	250	260	270	280	290	300
	DGKAPILGYAEDPVELFFMHIIQSGRLKTPSGKYIRIGYADKNEHPYVSI GRYMADKGYL					
m919.pep	310	320	330	340	350	360
	KLGQTSMQGIKSYMRQNPQRLAEVLGQNPSYI FFRELAGSSNDGPVGALGTPLMGEYAGA					
g919	310	320	330	340	350	360
	KLGQTSMQGIKAYMRQNPQRLAEVLGQNPSYI FFRELAGSGNEGPGVAGLTPLMGEYAGA					
m919.pep	370	380	390	400	410	420
	VDRHYITLGAPLFVATAHPVTRKALNRLIMAQDTGSAIKGAVRVDFWGYGDEAGELAGK					
g919	370	380	390	400	410	420
	IDRHYITLGAPLFVATAHPVTRKALNRLIMAQDTGSAIKGAVRVDFWGYGDEAGELAGK					
m919.pep	430	440				
	QKTTGYVWQLLPNGMKPEYRPX					
g919	430	440				
	QKTTGYVWQLLPNGMKPEYRPX					

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 38>:  
a919.seq

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1  ATGAAAAAAT ACCTATTCCG CGCCGCCCTG TGCGGCATCG CCGCCGCCAT
51  CCTCGCCGCC TGCCAAAGCA AGAGCATCCA AACCTTTCCG CAACCCGACA
101 CATCCGTCAT CAACGGCCCG GACCGGCCGG TCGGCATCCC CGACCCCGCC
151 GGAACGACGG TCGGCGGCGG CGGGGCCGTT TATACCGTTG TGCCGCACCT
201 GTCCCTGCCC CACTGGGCGG CGCAGGATT CGCCAAAAGC CTGCAATCCT
251 TCCGCTCGG CTGCGCCAAT TTGAAAAACC GCCAAGGCTG GCAGGATGTG
301 TGCGCCCAAG CCTTTCAAAC CCCCCTCCAT TCCGTTTCAGG CAAAACAGTT
351 TTTTGAACGC TATTTTACGC CGTGGCAGGT TGCAGGCAAC GGAAGCCTTG
401 CCGGTACGGT TACCGGCTAT TACGAGCCGG TGCTGAAGGG CGACGACAGG
451 CGGACGGCAC AAGCCCGCTT CCCGATTAC GGTATTCCCG ACGATTTTAT
501 CTCCGTCCCC CTGCCTGCCG GTTTGCGGAG CGGAAAAGCC CTTGTCCGCA
551 TCAGGCAGAC GGGAAAAAAC AGCGGCACAA TCGACAATAC CGGCGGCACA
601 CATACCGCCG ACCTCTCCCA ATTCCCCATC ACTGCGCGCA CAACGGCAAT
651 CAAAGGCAGG TTTGAAGGAA GCCGCTTCCT CCCCTACCAC ACGCGCAACC
701 AAATCAACGG CGGCGCGCTT GACGGCAAAG CCCCATACT CGGTTACGCC
751 GAAGACCCCG TCGAACTTTT TTTTATGCAC ATCCAAGGCT CGGGCCGTCT
801 GAAAACCCCG TCCGGCAAAT ACATCCGCAT CGGCTATGCC GACAAAAACG
851 AACATCCCTA CGTTTCCATC GGACGCTATA TGGCGGACAA AGGCTACCTC
901 AAGTCGGGC AGACCTCGAT GCAGGGCATC AAAGCCTATA TGCAGCAAAA
951 CCGCAACGC CTCGCCGAG TTTTGGGGCA AAACCCAGC TATATCTTTT
1001 TCCGAGAGCT TACCGGAAGC AGCAATGACG GCCCTGTCGG CGCACTGGGC
1051 ACGCCGCTGA TGGGCGAGTA CGCCGGCGCA GTCGACCGGC ACTACATTAC
1101 CTGGGCGCG CCCTTATTTG TCGCCACCGC CCATCCGGTT ACCCGCAAAG
1151 CCCTCAACCG CCTGATTATG GCGCAGGATA CCGGCAGCGC GATTAAGGC
1201 GCGGTGCGCG TGGATTATTT TTGGGGATAC GGCGACGAAG CCGGCGAACT
1251 TGCCGCAAA CAGAAAACCA CGGGATATGT CTGGCAGCTT CTGCCAACG
1301 GTATGAAGCC CGAATACCGC CCGTAA

```

This corresponds to the amino acid sequence <SEQ ID 39; ORF 919.a>:

```

a919.pep
1  MKKYLFRAL CGIAAAILAA CQSKSIQTFP QPDTSVINGP DRPVGIPDPA
51  GTTVGGGGAV YTVVPHLSLP HWAAQDFAKS LQSFRLGCAN LKNRQGWQDV
101 CAQAFQTPVH SVQAKQFFER YFTPWQVAGN GSLAGTVTGY YEPVLKGDDR
151 RTAQAQRFPIY GIPDDFISVP LPAGLRSGKA LVRIRQTGKN SGTIDNTGGT
201 HTADLSQFPI TARTTAIKGR FEGRSFLPYH TRNQINGGAL DGKAPILGYA
251 EDPVELFFMH IQGSGRLKTP SGKYIRIGYA DKNEHPYVSI GRYMADKGYL
301 KLGQTSMQGI KAYMQQNQR LAEVLGQNP YIFFRELTS SNGDPVGALG
351 TPLMGEYAGA VDRHYITLGA PLFVATAHPV TRKALNRLIM AQDTGSAIKG
401 AVRVDYFWGY GDEAGELAGK QKTTGYVWQL LPNGMKPEYR P*

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m919/a919 ORFs 919 and 919.a showed a 98.6% identity in 441 aa overlap

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m919.pep      10      20      30      40      50      60
MKKYLFRALYGI AAILAACQSKSIQTFPQPDTSVINGPDRPVGIPDPAGTTVGGGGAV
|||||
a919          10      20      30      40      50      60
MKKYLFRALCGI AAILAACQSKSIQTFPQPDTSVINGPDRPVGIPDPAGTTVGGGGAV
|||||

m919.pep      70      80      90      100     110     120
YTVVPHLSLPHW AAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
|||||
a919          70      80      90      100     110     120
YTVVPHLSLPHW AAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSVQAKQFFER
|||||

m919.pep     130     140     150     160     170     180
YFTPWQVAGNGSL AGTVTGYEYEPVLKGGDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
|||||
a919         130     140     150     160     170     180
YFTPWQVAGNGSL AGTVTGYEYEPVLKGGDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
|||||

m919.pep     190     200     210     220     230     240
LVRIRQTGKNSGT IDNTGGTHTADLSRFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
|||||

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a919      LVRI RQTG KNSGTIDNTGGTHTADLSQFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
           190      200      210      220      230      240

           250      260      270      280      290      300
m919.pep  DGKAPILGYAEDPVLEFFMHIIQSGSRLKTPSGKYIRIGYADKNEHPYVSIGRYMADKGYL
           |||||
a919      DGKAPILGYAEDPVLEFFMHIIQSGSRLKTPSGKYIRIGYADKNEHPYVSIGRYMADKGYL
           250      260      270      280      290      300

           310      320      330      340      350      360
m919.pep  KLGQTSMQGIKSYMQRNPQRLAEVLGQNPSYIFFRELAGSSNDGPVGALGTPLMGEYAGA
           |||||:|||||
a919      KLGQTSMQGIKAYMQQNPRQLAEVLGQNPSYIFFRELGTSSNDGPVGALGTPLMGEYAGA
           310      320      330      340      350      360

           370      380      390      400      410      420
m919.pep  VDRHYITLGAPLFVATAHPVTRKALNRLIMAQDTGSAIKGAVRVDYFWGYGDEAGELAGK
           |||||
a919      VDRHYITLGAPLFVATAHPVTRKALNRLIMAQDTGSAIKGAVRVDYFWGYGDEAGELAGK
           370      380      390      400      410      420

           430      440
m919.pep  QKTTGYVWQLLPNGMKPEYRPX
           |||||
a919      QKTTGYVWQLLPNGMKPEYRPX
           430      440

```

121 and 121-1

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 40>:

m121.seq

```

1  ATGGAAACAC AGCTTTACAT CGGCATCATG TCGGGAACCA GCATGGACGG
51  GGCGGATGCC GTACTGATAC GGATGGACGG CGGCAAATGG CTGGGCGCGG
101 AAGGGCACGC CTTTACCCCC TACCCCGGCA GGTTACGCCG CCAATTGCTG
151 GATTTGCAGG ACACAGGCGC AGACGAACTG CACGCGAGCA GGATTTTGTC
201 GCAAGAACTC AGCCGCCTAT ATGCGCAAAC CGCCGCCGAA CTGCTGTGCA
251 GTCAAAACCT CGCACCCTCC GACATTACCG CCCTCGGCTG CCACGGGCAA
301 ACCGTCCGAC ACGCGCCGGA ACACGGTTAC AGCATACAGC TTGCCGATTT
351 GCCGCTGCTG GCGxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx
401 xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx
451 xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx
501 xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx
551 xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx
601 xxxxxxCAGC TTCCTTACGA CAAAACGGT GCAAAGTCGG CACAAGGCAA
651 CATATTGCCG CAACTGCTCG ACAGGCTGCT CGCCACCCG TATTTGCGAC
701 AACGCCACCC TAAAGCACG GGGCGCGAAC TGTTTGCCAT AAATTGGCTC
751 GAAACCTACC TTGACGGCGG CGAAAACCGA TACGACGTAT TGCGGACGCT
801 TTCCCGTTTT ACCGCGCAA CCGTTTGCGA CGCCGTCTCA CACGACGCG
851 CAGATGCCCG TCAATGTAC ATTTGCGACG GCGGCATCCG CAATCCTGTT
901 TTAATGGCGG ATTTGGCAGA ATGTTTCGGC ACACGCGTTT CCCTGCACAG
951 CACGCGCGAC CTGAACCTCG ATCCGCAATG GGTGGAAGCC GCCGnATTG
1001 CGTGTTGGC GCGGTGTTGG ATTAATCGCA TTCCCGGTAG TCCGCACAAA
1051 GCAACCGGCG CATCAAACC GTGTATTCTG AnCGCGGGAT ATTATTATTG
1101 A

```

This corresponds to the amino acid sequence &lt;SEQ ID 41; ORF 121&gt;:

m121.pep

```

1  METQLYIGIM SGTSMDGADA VLIRMDGGKW LGAEGHAFTP YPGRLRRQLL
51  DLQDTGADEL HRSRILSQEL SRLYAQTAAE LLCSQNLAPS DITALGCHGQ

```

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```

101 TVRHAPEHGY SIQLADLPLL Axxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx
151 xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx
201 xxQLPYDKNG AKSAQGNILP QLLDRLLAHP YFAQRHPKST GRELFAINWL
251 ETYLDGGENR YDVLRTLRSF TAQTVCDAYS HAAADARQMY ICDGGIRNPV
301 LMADLAECFG TRVSLHSTAD LNLDPQWVEA AXFAWLAACW INRIPGSPHK
351 ATGASKPCIL XAGYYY*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 42>:

g121.seq

```

1 ATGGAACAC AGCTTTACAT CGGCATTATG TCGGGAACCA GTATGGACGG
51 GCGGATGCC GTGCTGGTAC GGATGGACGG CGGCAAATGG CTGGGCGCGG
101 AAGGGCACGC CTTTACCCCC TACCCTGACC GGTTCGCGCG CAAATTGCTG
151 GATTTGCAGG ACACAGGCAC AGACGAAGT CACCGCAGCA GGATGTTGTC
201 GCAAGAACTC AGCGCGCTGT ACAGCGCAAAC CGCGCGCGAA CTGCTGTGCA
251 GTCAAAACCT CGCTCCGTGC GACATTACCG CCCTCGGCTG CCACGGGCAA
301 ACCGTCCGAC ACAGCGCCGA ACACGGTtac AGCATAACAGC TTGCCGATTT
351 GCCGCTGCTG GCGGAAGTga cgcggatttt TACCGTCggc gacttcCGCA
401 GCCGCGACCT TGCTGCCGGC GGacaAGGTG CGCGCTCGT CCGCGCCTTT
451 CACGAAGCCC TGTTCCGCGA TGACAGGGAA ACACGCGTGG TACTGAACAT
501 CGCGGGGATT GCCAACATCA CGCTACTCCC CCGCGGCGCA CCGCGCTTCG
551 GCTTCGACAC AGGGCCGGGC AATATGCTGA TGGAcgcgtg gacgcaggca
601 cacTGGcagc TGCTTACGA CAAAacggt gcAAAGgcgg cacAAGGCAA
651 catatTGCcg cAACTGCTCG gcaggetGCT CGCCcaccCG TATTTCTCAC
701 AACCCcacc aaAAGCACG GGgcGCGaac Tgtttgccct AAattggctc
751 gaaacctAcc ttgacggcgg cgaaaaccga tacgacgtat tgcggacgct
801 ttcccgattc accgcgcaaA ccgTttggga cgcggtetca CACGCAGCGG
851 CAGATGCCCG TCAAATGTAC ATTTGCGGCG GCGGCATCCG CAATCCTGTT
901 TTAATGGCGG ATTTGGCAGA ATGTTTCGGC ACACGCGTTT CCCTGCACAG
951 CACCGCCGAA CTGAACCTCG ATCCTCAATG GGTGGAGGCG gccgCATTtg
1001 cgtggttgC GGCGTGTTGG ATTAACCGCA TTCCCGGTAG TCCGCACAAA
1051 GCGACCGGCG CATCAAACC GTGTATTCTG GGCGCGGGAT ATTATTATTG
1101 A

```

This corresponds to the amino acid sequence <SEQ ID 43; ORF 121.ng>:

g121.pep

```

1 METQLYIGIM SGTSMGDGADA VLVRMDGGKW LGAEGHAFTP YPDLRRKKLL
51 DLQDTGTDEL HRSRMLSQEL SRLYAQTAAE LLCSQNLAPC DITALGCHGQ
101 TVRHAPEHGY SIQLADLPLL AELTRIFTVG DFRSRDLAAG GQGAPLVPAF
151 HEALFRDDRE TRVVLNIGGI ANISVLPPGA PAFGFDTPG NMLMDAWTQA
201 HWQLPYDKNG AKAAQGNILP QLLGRLLAHP YFSQPHPKST GRELFALNWL
251 ETYLDGGENR YDVLRTLRSF TAQTVWDAVS HAAADARQMY ICGGGIRNPV
301 LMADLAECFG TRVSLHSTAE LNLDPQWVEA AAFAWLAACW INRIPGSPHK
351 ATGASKPCIL GAGYYY*

```

ORF 121 shows 73.5% identity over a 366 aa overlap with a predicted ORF (ORF121.ng) from *N. gonorrhoeae*:

m121/g121

	10	20	30	40	50	60
m121.pep	METQLYIGIMSGTSMGDGADAVLIRMDGGKWLGAEGHAFTPYPGRLRRQLLDLQDTGADEL					
	:     :     :     :     :					
g121	METQLYIGIMSGTSMGDGADAVLVRMDGGKWLGAEGHAFTPYPDLRRKKLLDLQDTGTDEL					
	10	20	30	40	50	60
	70	80	90	100	110	120
m121.pep	HRSRILSQELSRLYAQTAAELLCSQNLAPSDITALGCHGQTVRHAPEHGYSIQLADLPLL					
	:     :     :     :     :					
g121	HRSRMLSQELSRLYAQTAAELLCSQNLAPCDITALGCHGQTVRHAPEHGYSIQLADLPLL					
	70	80	90	100	110	120
	130	140	150	160	170	180

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```

m121.pep  AXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
          | : : : : : : : : : : : : : : : : : : : : : : :
g121      AELTRIFTVGDFRSRDLAAGGQGAPLVPFHEALFRDDRETRVVLNIGGIANISVLPPGA
          130      140      150      160      170      180
          190      200      210      220      230      240
m121.pep  XXXXXXXXXXXXXXXXXXXXQLPYDKNGAKSAQGNILPQLLDRLLAHPYFAQRHPKST
          : : : | | | | | : | | | | | | | | | | | | | | | |
g121      PAFGFDTGPGNMLMDAWTQAHWQLPYDKNGAKAAQGNILPQLLGRLLAHPYFSQPHPKST
          190      200      210      220      230      240
          250      260      270      280      290      300
m121.pep  GRELFAINWLETYLDGGENRYDVLRTLSRFTAQTVCDASHAAADARQMYICDGGIRNPV
          | | | | : | | | | | | | | | | | | | | | | | | | | |
g121      GRELFALNWLETYLDGGENRYDVLRTLSRFTAQTVWDASHAAADARQMYICGGGIRNPV
          250      260      270      280      290      300
          310      320      330      340      350      360
m121.pep  LMADLAECFGTRVSLHSTADLNLDPOWVEAAXFAWLAACWINRIPGSPHKATGASKPCIL
          | | | | | | | | | | : | | | | | | | | | | | | | | |
g121      LMADLAECFGTRVSLHSTAE LNLDPOWVEAAAFWLAACWINRIPGSPHKATGASKPCIL
          310      320      330      340      350      360

m121.pep  XAGYYYYX
          | | | |
g121      GAGYYYYX

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 44>:

```

a121.seq
1  ATGGAAACAC AGCTTTACAT CGGCATCATG TCGGGAACCA GCATGGACGG
51  GGCGGATGCC GTACTGATAC GGATGGACGG CGGCAATGG CTGGGCGCGG
101 AAGGGCACGC CTTACCCCC TACCCCGGCA GGTACGCGG CAAATTGCTG
151 GATTTGCAGG ACACAGGCGC GGACGAACTG CACCGCAGCA GGATGTTGTC
201 GCAAGAACTC AGCCGCTGT ACACGCAAC CGCCGCCGAA CTGCTGTGCA
251 GTCAAAACCT CGCGCCGTCC GACATTACCG CCCTCGGCTG CCACGGGCAA
301 ACCGTCAGAC ACACGCGCGA ACACAGTTAC AGCGTACAGC TTGCCGATTT
351 GCCGCTGCTG GCGGAACGGA CTCAGATTTT TACCGTCGGC GACTTCCGCA
401 GCCGCGACCT TCGCGCCGGC GGACAAGGCG CGCCGCTCGT CCCCGCCCTT
451 CACGAAGCCC TGTTCGCGA CGACAGGGAA ACACGCGCGG TACTGAACAT
501 CGGCGGGATT GCCAACATCA GCGTACTCCC CCCCAGCGCA CCGCCTTCG
551 GCTTCGACAC AGGACCGGGC AATATGCTGA TGGACGCGTG GATGCAGGCA
601 CACTGGCAGC TTCCTTACGA CAAAACGGT GCAAAGGCGG CACAAGGCAA
651 CATATTGCCG CAACTGCTCG ACAGGCTGCT CGCCCACCCG TATTTGCGAC
701 AACCACACCC TAAAGACAG GGGCGCGAAC TGTTCGCCCT AAATTGGCTC
751 GAAACCTACC TTGACGGCGG CGAAAACCGA TACGACGTAT TCGGGACGCT
801 TTCCCGATT ACCGCGCAA CCGTTTTCGA CGCCGTCTCA CACGCAGCGG
851 CAGATGCCCG TCAAATGTAC ATTTGCGGCG GCGGCATCCG CAATCCTGTT
901 TTAATGGCGG ATTTGGCAGA ATGTTTCGGC ACACGCGTTT CCCTGCACAG
951 CACCGCCGAA CTGAACCTCG ATCCGCAATG GGTAGAAGCC GCCGCGTTCC
1001 CATGGATGGC GCGGTGTTGG GTCAACCGCA TTCCCGGTAG TCCGCACAAA
1051 GCAACCGGCG CATCAAACC GTGTATTCTG GCGCGGGAT ATTATTATTG
1101 A

```

This corresponds to the amino acid sequence <SEQ ID 45; ORF 121.a>:

```

a121.pep
1  METQLYIGIM SGTSMDGADA VLIRMDGGKW LGAEGHAFTP YPGRLLRRKLL
51  DLQDTGADEL HRSRMLSQEL SRLYAQTAAE LLCSQNLAPS DITALGCHGQ
101 TVRHAPESY SVQLADLPLL AERTQIFTVG DFRSRDLAAG GQGAPLVPF
151 HEALFRDDRE TRAVLNIGGI ANISVLPPDA PAFGFDTGPG NMLMDAWMQA
201 HWQLPYDKNG AKAAQGNILP QLLDRLLAHP YFAQPHPKST GRELFALNWL
251 ETYLDGGENR YDVLRTLSRF TAQTVFDAYS HAAADARQMY ICGGGIRNPV
301 LMADLAECFG TRVSLHSTAE LNLDPOWVEA AAFWMAACW VNRIPGSPHK

```

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351 ATGASKPCIL GAGYYY\*

m121/a121 ORFs 121 and 121.a 74.0% identity in 366 aa overlap

m121.pep	10	20	30	40	50	60
	METQLYIGIMSGTSM	GADAVLIRMDGGKWL	GAEHGAFTPYPGRL	RRQLLDLQDTGADEL		
a121	10	20	30	40	50	60
	METQLYIGIMSGTSM	GADAVLIRMDGGKWL	GAEHGAFTPYPGRL	RRQLLDLQDTGADEL		
m121.pep	70	80	90	100	110	120
	HRSRILSQELSR	LYAQTAELLCSQNL	APSDITALGCHGQ	TVRHAPEHGYSIQ	LADLPLL	
a121	70	80	90	100	110	120
	HRSRILSQELSR	LYAQTAELLCSQNL	APSDITALGCHGQ	TVRHAPEHGYSIQ	LADLPLL	
m121.pep	130	140	150	160	170	180
	XXXXXXXXXXXXXXXX	XXXXXXXXXXXXXXXX	XXXXXXXXXXXXXXXX	XXXXXXXXXXXXXXXX	XXXXXXXXXXXXXXXX	XXXXXXXXXXXXXXXX
a121	130	140	150	160	170	180
	AERTQIFTVGDF	RSRDLAAGGQGA	PLVPAFHEALFR	DDRETRAVLNIG	GGIANISVLP	PDPA
m121.pep	190	200	210	220	230	240
	XXXXXXXXXXXXXXXX	XXXXXQLPYDKNG	AKSAQGNILPQL	LDRLLAHPYFAQ	RHPKST	
a121	190	200	210	220	230	240
	PAFGFDTGPGN	MLMDAWMQAHW	QLPYDKNGAKAA	QGNILPQLLDRL	LAHPYFAQPHK	ST
m121.pep	250	260	270	280	290	300
	GRELFAINWLE	TYLDGGENRYD	VLRTLSRFTAQ	TVCDVSHAAAD	AROMYICDGG	IRNPV
a121	250	260	270	280	290	300
	GRELFAINWLE	TYLDGGENRYD	VLRTLSRFTAQ	TVFVSHAAAD	AROMYICGGG	IRNPV
m121.pep	310	320	330	340	350	360
	LMADLAECF	GTRVSLHSTAD	NLDLPQWVEA	AXFAWLAACW	INRIPGSPHK	ATGASKPCIL
a121	310	320	330	340	350	360
	LMADLAECF	GTRVSLHSTAE	NLDLPQWVEA	AAFAWMAACW	VNRIIPGSPH	KATGASKPCIL
m121.pep						
	XAGYYYYX					
a121						
	GAGYYYYX					

Further work revealed the DNA sequence identified in *N. meningitidis* <SEQ ID 46>:

m121-1.seq

```

1  ATGGAACAC AGCTTTACAT CGGCATCATG TCGGGAACCA GCATGGACGG
51  GGCGGATGCC GTACTGATAC GGATGGACGG CGGCAATGG CTGGGCGCGG
101 AAGGGCACGC CTTTACCCCC TACCCCGGCA GGTACGCCG CCAATTGCTG
151 GATTTGCAGG ACACAGGCGC AGACGAACTG CACCGCAGCA GGATTTTGTC
201 GCAAGAACTC AGCCGCCTAT ATGCGCAAAC CGCCGCCGAA CTGCTGTGCA
251 GTCAAAACCT OGCACCGTCC GACATTACCG CCCTCGGCTG CCACGGGCAA
301 ACCGTCGAC ACGCGCCGGA ACACGGTTAC AGCATACAGC TTGCCGATTT
351 GCCGCTGCTG GCGGAACGGA CGCGGATTTT TACCGTCGGC GACTTCCGCA
401 GCCGCGACCT TCGCGCCGCG GGACAAGGCG CGCCACTCGT CCCCGCCTTT
451 CACGAAGCCC TGTTCGCGCA CAACAGGGAA ACACGCGCGG TACTGAACAT
501 CGGCGGGATT GCCAACATCA GCGTACTCCC CCCCACGCA CCCGCCTTCG
551 GCTTCGACAC AGGGCCGGGC AATATGCTGA TGGACGCGTG GACGCAGGCA
601 CACTGGCAGC TTCCTTACGA CAAAACGGGT GCAAAGGCGG CACAAGGCAA
651 CATATTGCCG CAACTGCTCG ACAGGCTGCT CGCCACCCG TATTTGCAC
701 AACCACCCC TAAAGCACG GGGCGCGAAC TGTTTGCCCT AAATTGGCTC
751 GAAACCTACC TTGACGCGCG CGAAAACCGA TACGACGTAT TGCGGACGCT

```



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```

801 TTCCCGTTTT ACCGCGCAAA CCGTTTGCGA CGCCGTCTCA CACGCAGCGG
851 CAGATGCCCG TCAAATGTAC ATTTGCGGCG GCGGCATCCG CAATCCTGTT
901 TTAATGGCGG ATTTGGCAGA ATGTTTCGGC ACACGCGTTT CCCTGCACAG
951 CACCGCCGAC CTGAACCTCG ATCCGCAATG GGTGGAAGCC GCCGNATTTG
1001 CGTGGTTGGC GCGGTGTTGG ATTAATCGCA TTCCCGGTAG TCCGCACAAA
1051 GCAACCGGCG CATCCAAACC GTGTATTCTG ANCGCGGGAT ATTATTATTG
1101 A

```

This corresponds to the amino acid sequence <SEQ ID 47; ORF 121-1>:

m121-1.pep

```

1  METQLYIGIM SGTSMDGADA VLIRMDGGKW LGAEGHAFTP YPGRLLRQLL
51  DLQDTGADEL HRSRILSQEL SRLYAQTAAE LLCSQNLAPS DITALGCHGQ
101 TVRHAPHEGY SIQLADLPLL AERTRIFTVG DFRSRDLAAG GGQAPLVPAF
151 HEALFRDNRE TRAVLNIGGI ANISVLPPDA PAFGFDTGPG NMLMDAWTQA
201 HWQLPYDKNG AKAAQGNILP QLLDRLLAHP YFAQPHPKST GRELFALNWL
251 ETYLDGGENR YDVLRTLSRF TAQTVCDAYS HAAADARQMY ICGGGIRNPV
301 LMADLAECFG TRVSLHSTAD LNLDPQWVEA AXFAWLAACW INRIPGSPHK
351 ATGASKPCIL XAGYYY*

```

m121-1/g121 ORFs 121-1 and 121-1.ng showed a 95.6% identity in 366 aa overlap

	10	20	30	40	50	60
m121-1.pep	METQLYIGIMSGTSMDGADAVLIRMDGGKW LGAEGHAFTPYPGRLLRQLLDLQDTGADEL					
g121	METQLYIGIMSGTSMDGADAVLVRMDGGKW LGAEGHAFTPYPDRLLRKLDDLQDTGTDEL					
	10	20	30	40	50	60
	70	80	90	100	110	120
m121-1.pep	HRSRILSQELSRLYAQTAAELLCSQNLAPSDITALGCHGQTVRHAPHEGYSIQLADLPLL					
g121	HRSRMLSQELSRLYAQTAAELLCSQNLAPCDITALGCHGQTVRHAPHEGYSIQLADLPLL					
	70	80	90	100	110	120
	130	140	150	160	170	180
m121-1.pep	AERTRIFTVGDFRSRDLAAGGGQAPLVPAFHEALFRDNRETRAVLNIGGIANISVLPPDA					
g121	AELTRIFTVGDFRSRDLAAGGGQAPLVPAFHEALFRDDRETRVVLNIGGIANISVLPPGA					
	130	140	150	160	170	180
	190	200	210	220	230	240
m121-1.pep	PAFGFDTGPGNMLMDAWTQAHWQLPYDKNGAKAAQGNILPQLLDRLLAHPYFAQPHPKST					
g121	PAFGFDTGPGNMLMDAWTQAHWQLPYDKNGAKAAQGNILPQLLGRLLAHPYFSQPHPKST					
	190	200	210	220	230	240
	250	260	270	280	290	300
m121-1.pep	GRELFALNWLETYLDGGENRYDVLRTLSRFTAQTVCDAYSHAAADARQMYICGGGIRNPV					
g121	GRELFALNWLETYLDGGENRYDVLRTLSRFTAQTVWDAYSHAAADARQMYICGGGIRNPV					
	250	260	270	280	290	300
	310	320	330	340	350	360
m121-1.pep	LMADLAECFGTRVSLHSTADLNLDPQWVEAAXFAWLAACWINRIPGSPHKATGASKPCIL					
g121	LMADLAECFGTRVSLHSTAELNLDPQWVEAAAFWLAACWINRIPGSPHKATGASKPCIL					
	310	320	330	340	350	360
m121-1.pep	XAGYYYX					
g121	GAGYYYX					

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The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 48>:

```
a121-1.seq
1  ATGGAAACAC AGCTTTACAT CGGCATCATG TCGGGAACCA GCATGGACGG
51  GGCGGATGCC GTACTGATAC GGATGGACGG CGGCAAATGG CTGGGCGCGG
101 AAGGGCACGC CTTTACCCCC TACCCCGGCA GGTACGCCG CAAATTGCTG
151 GATTTCAGG ACACAGGCGC GGACGAACTG CACCGCAGCA GGATGTTGTC
201 GCAAGAACTC AGCCGCCTGT ACGCGCAAAC CGCCGCCGAA CTGCTGTGCA
251 GTCAAAACCT CGCGCCGTCC GACATTACCG CCCTCGGCTG CCACGGGCAA
301 ACCGTCAGAC ACGCGCCGGA ACACAGTTAC AGCGTACAGC TTGCCGATTT
351 GCCGTGCTG GCGGAACGGA CTCAGATTTT TACGTCGGC GACTTCCGCA
401 GCCGCGACCT TCGCGCCGGC GGACAAGGCG CGCCGCTCGT CCCCGCCTTT
451 CACGAAGCCC TGTTCCGCGA CGACAGGGAA ACACGCGCGG TACTGAACAT
501 CGGCGGGATT GCCAACATCA GCGTACTCCC CCCCACGCA CCCGCCTTCG
551 GCTTCGACAC AGGACCGGGC AATATGCTGA TGGACCGGTG GATGCAGGCA
601 CACTGGCAGC TTCCTTACGA CAAAACGGT GCAAAGGCGG CACAAGGCAA
651 CATATTGCCG CAACTGCTCG ACAGGCTGCT CGCCCACCGG TATTTCGCAC
701 AACCCACACC TAAAAGCAGC GGGCGCGAAC TGTTTGCCCT AAATTGGCTC
751 GAAACCTACC TTGACGGCGG CGAAAACCGA TACGACGTAT TGCGGACGCT
801 TTCCCGATTG ACCCGCAAAA CCGTTTTCGA CGCCGTCTCA CACGCAGCGG
851 CAGATGCCCG TCAAATGTAC ATTTGCGGCG GCGGCATCCG CAATCCTGTT
901 TTAATGGCGG ATTTGGCAGA ATGTTTCGGC ACACGCGTTT CCCTGCACAG
951 CACCGCCGAA CTGAACCTCG ATCCGCAATG GGTAGAAGCC GCCGCGTTCG
1001 CATGGATGGC GCGGTGTTGG GTCAACCGCA TTCCCGGTAG TCCGCACAAA
1051 GCAACCGGCG CATCCAAACC GTGTATTCTG GCGCGGGAT ATTATTATTG
1101 A
```

This corresponds to the amino acid sequence <SEQ ID 49; ORF 121-1.a>:

```
a121-1.pep
1  METQLYIGIM SGTSMGDADA VLIRMDGGKW LGAEGHAFTP YPGRLLRRKLL
51  DLQDTGADEL HRSRMLSQEL SRLYAQTAAE LLCQNLAAPS DITALGCHGQ
101 TVRHAPESY SVQLADLPLL AERTQIFTVG DFRSRDLAAG GQGAPLVPAF
151 HEALFRDDRE TRAVLNIGGI ANISVLPPDA PAFGFDTPGP NMLMDAWMQA
201 HWQLPYDKNG AKAAQGNILP QLLDRLLAHP YFAQPHPKST GRELFALNWL
251 ETYLDGGENR YDVLRTLRF TAQTVFDAVS HAAADARQMY ICGGGIRNPV
301 LMADLAECFG TRVSLHSTAE LNLDPQWVEA AFAWMAACW VNRIPGSPHK
351 ATGASKPCIL GAGYYY*
```

m121-1/a121-1 ORFs 121-1 and 121-1.a showed a 96.4% identity in 366 aa overlap

	10	20	30	40	50	60
m121-1.pep	METQLYIGIMSGTSMGDADAVLIRMDGGKWLGAEGHAFTPPYPGRLLRRQLLDLQDTGADEL					
a121-1	METQLYIGIMSGTSMGDADAVLIRMDGGKWLGAEGHAFTPPYPGRLLRRQLLDLQDTGADEL					
	10	20	30	40	50	60
m121-1.pep	HRSRILSQELSRSLYAQTAAELLCSQNLAAPSDITALGCHGQTVRHAPHEHGYSIQLADLPLL					
a121-1	HRSRMLSQELSRSLYAQTAAELLCSQNLAAPSDITALGCHGQTVRHAPESYSVQLADLPLL					
	70	80	90	100	110	120
m121-1.pep	AERTQIFTVGDFRSRDLAAGGQGAPLVPAFHEALFRDNRETRAVLNIGGIANISVLPPDA					
a121-1	AERTQIFTVGDFRSRDLAAGGQGAPLVPAFHEALFRDDRETRAVLNIGGIANISVLPPDA					
	130	140	150	160	170	180
m121-1.pep	PAFGFDTPGPNMLMDAWTQAHWQLPYDKNGAKAAQGNILPQLLDRLLAHPYFAQPHPKST					
a121-1	PAFGFDTPGPNMLMDAWMQAHWQLPYDKNGAKAAQGNILPQLLDRLLAHPYFAQPHPKST					
	190	200	210	220	230	240
m121-1.pep	PAFGFDTPGPNMLMDAWTQAHWQLPYDKNGAKAAQGNILPQLLDRLLAHPYFAQPHPKST					
a121-1	PAFGFDTPGPNMLMDAWMQAHWQLPYDKNGAKAAQGNILPQLLDRLLAHPYFAQPHPKST					

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	250	260	270	280	290	300
m121-1.pep	GRELFAFNWLETYLDGGENRYDVLRTLSRFTAQTVCDASHAAADARQMYICGGGIRNPV					
a121-1	GRELFAFNWLETYLDGGENRYDVLRTLSRFTAQTVFDAVSHAAADARQMYICGGGIRNPV					
	250	260	270	280	290	300
	310	320	330	340	350	360
m121-1.pep	LMADLAECFGRVSLHSTADLNLDQWVEAAXFAWLAACWINRIPGSPHKATGASKPCIL					
a121	LMADLAECFGRVSLHSTAEENLDQWVEAAAFWMAACWVNRIPGSPHKATGASKPCIL					
	310	320	330	340	350	360
m121-1.pep	XAGYYYYX					
a121	GAGYYYYX					

128 and 128-1

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 50>:

```

m128.seq (partial)
1  ATGACTGACA  ACGCACTGCT  CCATTTGGGC  GAAGAACCCC  GTTTTGATCA
51  AATCAAAACC  GAAGACATCA  AACCCGCCCT  GCAAACCGCC  ATCGCCGAAG
101 CGCGCGAACA  AATCGCCGCC  ATCAAAGCCC  AAACGCACAC  CGGCTGGGCA
151 AACACTGTCG  AACCCTGAC  CGGCATCACC  GAACGCGTCG  GCAGGATTTG
201 GGGCGTGGTG  TCGCACCTCA  ACTGCGTCGC  CGACACGCCC  GAACTGCGCG
251 CCGTCTATAA  CGAACTGATG  CCCGAAATCA  CCGTCTTCTT  CACCGAAATC
301 GGACAAGACA  TCGAGCTGTA  CAACCGCTTC  AAAACCATCA  AAAATTCCCC
351 CGAATTCGAC  ACCCTCTCCC  CCGCACAAA  AACCAACTC  AACCAC
1  TACGCCAGCG  AAAAAGTGGC  CGAAGCCAAA  TACGCGTTCA  GCGAAACCGA
51  wGTCAAAAA  TAYTTCCTCG  TCGGCAAwGT  ATTAACCGGA  CTGTTTCGCCC
101 AAmTCAAAAA  ACTmTACGGC  ATCGGATTTA  CCGAAAAAAC  yGTCCCCGTC
151 TGGCACAAG  ACGTGCCTA  TTKTGAATTG  CAACAAAACG  GCGAAmCCAT
201 AGGCGGCGTT  TATATGGATT  TGTACGCACG  CGAAGGCAA  CGCGGCGGCG
251 CGTGGATGAA  CGACTACAAA  GGCCGCCGCC  GTTTTTCAGA  CGGCACGCTG
301 CAAyTGCCCA  CCGCTACCT  CGTCTGCAAC  TTCGCCCCAC  CCGTCCGCGG
351 CAGGGAAGCC  CGCyTGAGCC  ACGACGAAAT  CCTCATCCTC  TTCCACGAAA
401 CCGGACACGG  GCTGCACCAC  CTGCTTACCC  AAGTGGACGA  ACTGGGCGTA
451 TCCGGCATCA  ACGGCGTAKA  ATGGGACGCG  GTCGAACTGC  CCAGCCAGTT
501 TATGGAATAT  TTCGTTTGGG  AATACAATGT  CTTGGCACA  mTGTTCAGCCC
551 ACGAAGAAAC  CGGcgTTCCC  yTGCCGAAAG  AACTCTTsGA  CAAAwTGCTC
601 GCCGCCAAAA  ACTTCCAAsG  CGGCATGTTC  yTsGTCCGGC  AAwTGGAGTT
651 CGCCCTCTTT  GATATGATGA  TTTACAGCGA  AGACGACGAA  GGCCGTCTGA
701 AAAACTGGCA  ACAGGTTTTA  GACAGCGTGC  GCAAAAAAGT  CGCCGTCTATC
751 CAGCCGCCCG  AATACAACCG  CTTGCGCTTG  AGCTTCGGCC  ACATCTTCGC
801 AGGCGGCTAT  TCCGCAGCTn  ATTACAGCTA  CGCGTGGGCG  GAAGTATTGA
851 GCGCGGACGC  ATACGCCGCC  TTTGAAGAAA  GCGACGATGT  CGCCGCCACA
901 GGCAAACGCT  TTTGGCAGGA  AATCCTCGCC  GTCGGGgNat  CGCGCAGCGG
951 nGCAGAATCC  TTCAAAGCCT  TCCGCGGCCG  CGAACCGAGC  ATAGACGCAC
1001 TCTTGCGCCA  CAGCGGTTTC  GACAACGCGG  TCTGA

```

This corresponds to the amino acid sequence &lt;SEQ ID 51; ORF 128&gt;:

```

m128.pep (partial)
1  MTDNALLHLG  EEPRFDQIKT  EDIKPALQTA  IAEAREQIAA  IKAQHTTGWA
51  NTVEPLTGIT  ERVGRWGVV  SHLNCVADTP  ELRAVYNELM  PEITVFFTEI
101 GQDIELYNRF  KTIKNSPEFD  TLSPAQTKL  NH
//
1  YASEKLREAK  YAFSETXVKK  YFPVGXVLNG  LFAQXKKLYG  IGFTKTPVP

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```

51  WHKDVRYXEL QQNGEXIGGV YMDLYAREGK RGGAWMNDYK GRRRFSDBGTL
101  QLPTAYLVCN FAPPVGGREA RLSHDEILIL FHETGHGLHH LLTQVDELGV
151  SGINGVXWDA VELPSQFMEN FVWEYNVLAQ XSAHEETGVP LPKELXDKXL
201  AAKNFQXGMF XVRQXEFALF DMMIYSEDDE GRLKNWQQVL DSVRKKVAVI
251  QPPEYNRFAL SFGHIFAGGY SAAXYSYAWA EVLSADAYAA FEESDDVAAT
301  GKRFWQEILA VGXSRSGAES FKAFRGREPS IDALLRHS GF DNAV*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 52>:

```

g128.seq
1   atgattgaca acgCActgct ccacttgggc gaagaaccCC GTTTaatca
51  aatccaaacc gaagACatca AACCCGCCGT CCAAACCGCC ATCGCCGAAG
101 CGCGCGGACA AATCGCCGCC GTCAAAGCGC AAACGCACAC CGGCTGGGCG
151 AACACCGTCG AGCGTCTGAC CGGCATCACC GAACGCGTCG GCAGGATTGT
201 GGGCGTCGTG TCCCATCTCA ACTCCGTCGT CGACACGCCC GAACTGCGCG
251 CCGTCTATAA CGAACTGATG CCTGAAATCA CCGTCTTCTT CACCGAAATC
301 GGACAAGACA TCGAACTGTA CAACCGCTTC AAAACCATCA AAAATTCCCC
351 CGAATTTGCA ACGCTTTCCC CCGCACAAAA AACCAAGCTC GATCACGACC
401 TCGCGGATTT CGTATTGAGC GCGCGGGAAC TGCCGCCCGA ACGGCAGGCA
451 GAACTGGCAA AACTGCAAAAC CGAAGGCGCG CAACTTTCCG CCAAATTCTC
501 CCAAAACGTC CTAGACGCGA CCGACGCGTT CGGCATTAC TTTGACGATG
551 CCGCACCGCT TGCGGCATT CCCGAAGACG CGCTCGCCAT GTTTGCCGCC
601 GCCGCGCAAA GCGAAGGCAA AACAGGTTAC AAAATCGGCT TGCAGATTCC
651 GCACTACCTT GCCGTTATCC AATACGCGG CAACCGCGAA CTGCGCGAAC
701 AAATCTACCG CGCTACGTT ACCCGTGCCA GCGAACTTTC AAACGACGGC
751 AAATTCGACA ACACCGCCAA CATCGACCGC ACGCTCGAAA ACGCATTGAA
801 AACCGccaaa cTGCTCGGCT TAAAAATTA CGCCGAATTG TCGCTGGCAA
851 CCAAAATGGC GGACACGCC GAACAGTTT TAAACTTCCT GCACGACCTC
901 GCCCGCCGCG CCAAACCCTA CGCCGAAAAA GACCTCGCCG AAGTCAAAGC
951 CTTCCGCCCG GAACACCTCG GTCTCGCCGA CCCGCAGCCG TGGGACTTGA
1001 GCTACGCCGG GAAAAAACTG CGCGAAGCCA AATACGCATT CAGCGAAACC
1051 GAAGTCAAAA AATACTTCCC CGTCGGCAAA GTTCTGGCAG GCCTGTTTCG
1101 CCAAAATCAA AACTCTACG GCATCGGATT CGCCGAAAAA ACCGTTCCCG
1151 TCTGGCACAA AGACGTGCGC TATTTTGAAT TGCAACAAAA CGGCAAAACC
1201 ATCGGCGGCG TTTATATGGA TTTGTACGCA CGCGAAGGCA AACCGCGCGG
1251 CGCGTGGATG AACGActaca AAGGCCGCCG CCGCTTTGCC GACGgcacGC
1301 TGCAACTGCC CACCGCCTAC CTCGTCTGCA ACTTCGCCCC GCCCGTCGGC
1351 GGCAAAGAAG OGCGTTTAAG CCACGACGAA ATCCTCACCC TCTTCCACGA
1401 AacCGGCCAC GGACTGCACC ACCTGCTTAC CCAAGTGGAC GAACTGGGCG
1451 TGTCCGGCAT CAacggcgta GAATGGGACG CGGTGGAAT GCCCGACG
1501 TTTATGGAAA ACTTCGTTTG GGAATACAAT GTATTGGCAC AAATGTCCGC
1551 CCACGAAGAA AccgGCGAGC CCCTGCCGAA AGAACTCTTC GACAAAATGC
1601 TcgCGCCAA AAATTTCCAG CGCGGTATGT TCCTCGTCCG GCAAATGGAG
1651 TTCCGCCCTC TCGATATGAT GATTTACAGT GAAAGCGACG AATGCCGTCT
1701 GAAAAACTGG CAGCAGGTTT TAGACAGCGT GCGCAAAGAA GTcGCCGTCA
1751 TCCAACCGCC CGAATACAAC CGCTTCGCCA ACAGCTTCGG CCacatctTC
1801 GCcggcGGCT ATTCCGCGAG CTATTACAGC TACGCATGGG CCGAAGTCCT
1851 cAGCACCGAT GCCTACGCCG CCTTTGAAGA AAGcGACGac gtcGCCGCCA
1901 CAGGCAAACG CTTCTGGCAA GAAAtccttg ccgtcggcgg ctCCCGCAGC
1951 gcgCGGGAAT CCTTCAAAGC CTTCCGCGGA CGCGAACC GAATAGACGC
2001 ACTGCTGCGC CAaagcggtT TCGACAACGC gGcttgA

```

This corresponds to the amino acid sequence <SEQ ID 53; ORF 128.ng>:

```

g128.pep
1   MIDNALLHLG EEPRFNQIQT EDIKPAVQTA IAEARGQIAA VKAQHTTGWA
51  NTVERLTGIT ERVGRIWGVV SHLNSVVDTP ELRAVYNELM PEITVFFTEI
101 GQDIELYNRF KTIKNSPEFA TLSPAQKTKL DHDLRDFVLS GAELPPERQA
151 ELAKLQTEGA QLSAKFSQNV LDATDAFGIY FDDAAPLAGI PEDALAMFAA
201 AAQSEGKTYG KIGLQIPHYL AVIQYAGNRE LREQIYRAYV TRASELSNDG

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251 KFDNTANIDR TLENALKTAK LLGFKNYAEL SLATKMADTP EQVLNFLHDL
301 ARRAKPYAEK DLAEVKAFAR EHLGLADPQP WDL SYAGEKL REAKYAFSET
351 EVKKYFPVGK VLAGLFAQIK KLYGIGFAEK TVPVWHKDVR YFELQONGKT
401 IGGVYMDLYA REGKRGGAWM NDYKGRRRFA DGTLLQLPTAY LVCNFAPPVG
451 GKEARLSHDE ILTLFHETGH GLHHLLTQVD ELGVSGINGV EWDAVELPSQ
501 FMENFVWEYN VLAQMSAHEE TGEPLPKELF DKMLAANKFQ RGMFLVRQME
551 FALFDMMIYS ESDECRLKNW QQVLDSVRKE VAVIQPPEYN RFANSFGHIF
601 AGGYSAGYYS YAWAEVLSTD AYAAFEESDD VAATGKRFWQ EILAVGGSRS
651 AAESFKAFRG REPSIDALLR QSGFDNAA*

```

ORF 128 shows 91.7% identity over a 475 aa overlap with a predicted ORF (ORF 128.ng) from *N. gonorrhoeae*:

m128/g128

```

      10      20      30      40      50      60
g128.pep  MIDNALLHLGEEPRFNQIQTEDIKPAVQTAIAEARGQIAAVKAQTHGTWANTVERLTGIT
          | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
m128      MTDNALLHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAAIKAQTHGTWANTVEPLTGIT
          | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
      10      20      30      40      50      60

      70      80      90     100     110     120
g128.pep  ERVGRIWGVVSHLNSVVDTPELRAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFA
          | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
m128      ERVGRIWGVVSHLNCVADTPELRAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD
          | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
      70      80      90     100     110     120

      130     140     150     160     170     180
g128.pep  TLSPAQKTKLDHDLRDFVLSGAELPPERQAELAKLQTEGAQLSAKFSQNVLDATDAFGIY
          | | | | | | | | | |
m128      TLSPAQKTKLNH
          |
          130
              //

                                340     350     360
g128.pep  YAGEKLREAKYAFSETEVKKYFPVGKVLNG
          | | | | | | | | | | | | | | | | | |
m128      YASEKLREAKYAFSETXVKKYFPVGXVLNG
                                10      20      30

      370     380     390     400     410     420
g128.pep  LFAQIKKLYGIGFAEKTVPVWHKDVR YFELQONGKTIGGVYMDLYAREGKRGGAWMNDYK
          | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
m128      LFAQXKKLYGIGFTEKTPVWHKDVR YXELQONGEXIGGVYMDLYAREGKRGGAWMNDYK
          | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
          40      50      60      70      80      90

      430     440     450     460     470     480
g128.pep  GRRRFADGTLQLPTAYLVCNFAPPVGGKEARLSHDEILTLFHETGHGLHHLLTQVDELGV
          | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
m128      GRRRFSDGTLQLPTAYLVCNFAPPVGGREARLSHDEILILFHETGHGLHHLLTQVDELGV
          | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
          100     110     120     130     140     150

      490     500     510     520     530     540
g128.pep  SGINGVEWDAVELPSQFMENFVWEYNVLAQMSAHEETGEPLPKELFDKMLAANKFQRMGMF
          | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
m128      SGINGVXWDAVELPSQFMENFVWEYNVLAQXSAHEETGVPLPKELXDKMLAANKFQXGMF
          | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
          160     170     180     190     200     210

      550     560     570     580     590     600

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g128.pep	LVRQMEFALFDMMIYSEDECRLKNWQQVLDVSRKEVAVIQPPEYNRFANSFGHIFAGGY
m128	XVRQXEFALFDMMIYSEDEGRLLKNWQQVLDVSRKKVAVIQPPEYNRFALSFGHIFAGGY
	220 230 240 250 260 270
g128.pep	610 620 630 640 650 660
	SAGYYSYAWAEVLSTDAYAAFEESDDVAATGKRFWQEILAVGGSRSAAESFKAFRGREPS
m128	SAAXYSYAWAEVLSADAYAAFEESDDVAATGKRFWQEILAVGXSRSAGAESFKAFRGREPS
	280 290 300 310 320 330
g128.pep	670 679
	IDALLRQSGFDNAAX
m128	IDALLRHSFGFDNAVX
	340

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 54>:

a128.seq

1	ATGACTGACA	ACGCACTGCT	CCATTTGGGC	GAAGAACCCC	GTTTTGATCA
51	AATCAAAACC	GAAGACATCA	AACCCGCCCT	GCAAACCGCC	ATTGCCGAAG
101	CGCGCGAACA	AATCGCCGCC	ATCAAAGCCC	AAACGCACAC	CGGCTGGGCA
151	AACACTGTCTG	AACCCCTGAC	CGGCATCACC	GAACGCGTCG	GCAGGATTTG
201	GGGCGTGGTG	TCGCACCTCA	ACTCCGTCAC	CGACACGCCC	GAACGCGCG
251	COGCCTACAA	TGAATTAATG	CCCGAAATTA	CCGTCTTCTT	CACCGAAATC
301	GGACAAGACA	TCGAGCTGTA	CAACCGCTTC	AAAACCATCA	AAAACCTCCC
351	CGAGTTCGAC	ACCCTCTCCC	ACGCGCAAAA	AACCAAATC	AACCACGATC
401	TGCGCGATTT	CGTCCTCAGC	GGCGCGGAAC	TGCCGCCCGA	ACAGCAGGCA
451	GAATTGGCAA	AACGCAAAAC	CGAAGGCGCG	CAACTTCCG	CCAAATTC
501	CCAAACGTC	CTAGACGCGA	CCGACGCGT	CGGCATTAC	TTTGACGATG
551	CCGCACCGCT	TGCCGCGATT	CCCGAAGACG	CGCTCGCCAT	GTTTGCCGCT
601	GCCGCGCAAA	GCGAAGGCAA	AACAGGCTAC	AAAATCGGTT	TGCAGATTCC
651	GCACTACCTC	GCCGTACATC	AATACGCCGA	CAACCGCAAA	CTGCGCGAAC
701	AAATCTACCG	CGCCTACGTT	ACCCGCGCCA	GCGAGCTTTC	AGACGACGGC
751	AAATTCGACA	ACACCGCCAA	CATCGACCGC	ACGCTCGAAA	ACGCCCTGCA
801	AACCGCCAAA	CTGCTCGGCT	TCAAAAACCTA	CGCCGAATTG	TCGCTGGCAA
851	CCAAATGGC	GGACACCCCC	GAACAAGTTT	TAAACTTCCT	GCACGACCTC
901	GCCCGCCGCG	CCAAACCTTA	CGCCGAAAAA	GACCTCGCCG	AAGTCAAAGC
951	CTTCGCCCGC	GAAAGCCTCG	GCCTCGCCGA	TTTGCAACCG	TGGGACTTGG
1001	GCTACGCCGG	CGAAAACTG	CGCGAAGCCA	AATACGCATT	CAGCGAAACC
1051	GAAGTCAAAA	AATACTTCCC	CGTCGGCAAA	GTATTAAACG	GACTGTTCCG
1101	CCAAATCAAA	AAACTCTACG	GCATCGGATT	TACCGAAAAA	ACCGTCCCCG
1151	TCTGGCACAA	AGACGTGCGC	TATTTTGAAT	TGCAACAAAA	CGGCGAAACC
1201	ATAGGCGGCG	TTTATATGGA	TTTGTACGCA	CGCGAAGGCA	AACGCGGCGG
1251	CGCGTGGATG	AACGACTACA	AAGGCCGCGG	CCGTTTTTCA	GACGGCACGC
1301	TGCAACTGCC	CACCGCCTAC	CTCGTCTGCA	ACTTCACCCC	GCCCGTCGGC
1351	GGCAAAGAAG	CCCGCTTGAG	CCATGACGAA	ATCCTCACCC	TCTTCCACGA
1401	AACCGGACAC	GGCCTGCACC	ACCTGCTTAC	CCAAGTCGAC	GAACGCGGCG
1451	TATCCGGCAT	CAACGGCGTA	GAATGGGACG	CAGTCGAACT	GCCCAGTCAG
1501	TTTATGGAAA	ATTCGTTTG	GGAATACAAT	GTCTTGGCGC	AAATGTCCGC
1551	CCACGAAGAA	ACCGGCGTTC	CCCTGCCGAA	AGAACTCTTC	GACAAAAATG
1601	TCGCGGCCAA	AAACTTCCAA	CGCGGAATGT	TCCTCGTCCG	CCAAATGGAG
1651	TTCCGCCCTCT	TTGATATGAT	GATTTACAGC	GAAGACGACG	AAGGCCGTCT
1701	GAAAACTGG	CAACAGGTTT	TAGACAGCGT	GCGCAAAGAA	GTCGCCGTCG
1751	TCCGACCGCC	CGAATACAAC	CGCTTCGCCA	ACAGCTTCGG	CCACATCTTC
1801	GCAGGCGGCT	ATTCCGCAGG	CTATTACAGC	TACGCGTGGG	CGGAAGTATT
1851	GAGCGCGGAC	GCATACGCCG	CCTTTGAAGA	AAGCGACGAT	GTCGCCGCCA
1901	CAGGCAAACG	CTTTTGGCAG	GAAATCCTCG	CCGTCGGCGG	ATCGCGCAGC
1951	GCGGCAGAA	CCTTCAAAGC	CTTCCGCGGA	CGCGAACCGA	GCATAGACGC
2001	ACTCTTGCGC	CACAGCGGCT	TCGACAACGC	GGCTTGA	

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This corresponds to the amino acid sequence <SEQ ID 55; ORF 128.a>:

```

a128.pep
1   MTDNALLHLG EEPFRDQIKT EDIKPALQTA IAEAREQIAA IKAQHTGTGWA
51  NTVEPLTGIT ERVGRIWGVV SHLNSVTDTP ELRAAYNELM PEITVFFTEI
101 GQDIELYNRF KTIKNSPEFD TLSHAQKTKL NHDLRDFVLS GAELPPEQQA
151 ELAKLQTEGA QLSAKFSQNV LDATDAFGIY FDDAAPLAGI PEDALAMFAA
201 AAQSEGKTGY KIGLQIPHYL AVIQYADNRK LREQIYRAYV TRASELSDDG
251 KFDNTANIDR TLENALQTAK LLGFKNYAEL SLATKMADTP EQVLNFLHDL
301 ARRAKPYAEK DLAEVKAFAR ESLGLADLOP WDLGYAGEKL REAKYAFSET
351 EVKKYFPVGK VLNGLFAQIK KLYGIGFTEK TVPVWHKDVR YFELQONGET
401 IGGVYMDLYA REGKRGGAWM NDYKGRRRFS DGTQLQPTAY LVCNFTPPVG
451 GKEARLSHDE ILTLFHETGH GLHLLTQVD ELGVSGINGV EWDVLELRSO
501 FMENFVWEYN VLAQMSAHEE TGVPLPKELF DKMLAANKFQ RGMFLVRQME
551 FALFDMMIYS EDDEGRLEKNW QVLDLVRKE VAVVRPEYN RFANSFGHIF
601 AGGYSAGYYS YAWAEVLSAD AYAAFEESDD VAATGKRFQW EILAVGGSRS
651 AAESFKAFRG REPSIDALLR HSGFDNAA*

```

m128/a128 ORFs 128 and 128.a showed a 66.0% identity in 677 aa overlap

```

m128.pep      10      20      30      40      50      60
MTDNALLHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAAIIKAQHTGTGWANTVEPLTGIT
|||||
a128           10      20      30      40      50      60
MTDNALLHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAAIIKAQHTGTGWANTVEPLTGIT

m128.pep      70      80      90      100     110     120
ERVGRIWGVVSHLNCVADTPELRAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD
|||||:|||||:|||||:|||||:|||||:|||||
a128           70      80      90      100     110     120
ERVGRIWGVVSHLNSVTDTPELRAAYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD

m128.pep      130
TLSPAQKTKLNH-----
||| |||||
a128           130     140     150     160     170     180
TLSHAQKTKLNHDLRDFVLSGAELPPEQQAELAKLQTEGAQLSAKFSQNVLDATDAFGIY

m128.pep      -----
a128           190     200     210     220     230     240
FDDAAPLAGIPEDALAMFAAAAQSEGKTGYKIGLQIPHYLAVIQYADNRKLRREQIYRAYV

m128.pep      -----
a128           250     260     270     280     290     300
TRASELSDDGKFDNTANIDRTLENALQTAKLLGFKNYAELSLATKMADTPEQVLNFLHDL

m128.pep      140     150
-----YASEKLREAKYAFSETXVKKYFPVGX
||:|||||:|||||
a128           310     320     330     340     350     360
ARRAKPYAEKDLAEVKAFARESLGLADLOPWLGYAGEKLREAKYAFSETEVKKYFPVGK

m128.pep      160     170     180     190     200     210
VLNGLFAQXKKLYGIGFTEKTVPVWHKDVRXYELQONGEXIGGVYMDLYAREGKRGGAWM
|||||:|||||:|||||:|||||:|||||:|||||
a128           370     380     390     400     410     420
VLNGLFAQIKKLYGIGFTEKTVPVWHKDVRXYFELQONGEXIGGVYMDLYAREGKRGGAWM

m128.pep      220     230     240     250     260     270
NDYKGRRRFSDGTQLQPTAYLVCNFAPPVGGREARLSHDEILILFHETGHGLHLLTQVD

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```

|||||:|||||:|||||:|||||:|||||
a128      NDYKGRRRFSDGTLQLPTAYLVCNFTPPVGGKEARLSHDEILTLFHETGHLHLLTQVD
           430      440      450      460      470      480

m128.pep  280      290      300      310      320      330
           ELGVSGINGVXWDAVELPSQFMENFVWEYNVLAQXSAHEETGVPLPKELXDKXLAAKNFQ
           |||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
a128      ELGVSGINGVEWDAVELPSQFMENFVWEYNVLAQMSAHEETGVPLPKELFDKMLAAKNFQ
           490      500      510      520      530      540

m128.pep  340      350      360      370      380      390
           XGMFXVRQXEFAFDMMIYSEDDEGR LKNWQVLD SVRKKVAVIQPPEYNRFALSFGHIF
           ||| ||| |||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
a128      RGMFLVRQMEFAFDMMIYSEDDEGR LKNWQVLD SVRKEVAVVVRPPEYNRFANSFGHIF
           550      560      570      580      590      600

m128.pep  400      410      420      430      440      450
           AGGYSAAXYSYAWAEVLSADAYAAFEESDDVAATGKRFWQEILAVGXSRSGAESFKAFRG
           |||||: |||||: |||||: |||||: |||||: |||||: |||||: |||||: |||||
a128      AGGYSAGYYSYAWAEVLSADAYAAFEESDDVAATGKRFWQEILAVGGSRSAAESFKAFRG
           610      620      630      640      650      660

m128.pep  460      470
           REPSIDALLRHSGFDNAVX
           |||||:
a128      REPSIDALLRHSGFDNAAX
           670

```

Further work revealed the DNA sequence identified in *N. meningitidis* <SEQ ID 56>:

```

m128-1.seq
1   ATGACTGACA ACGCACTGCT CCATTGGGC GAAGAACCCC GTTTTGATCA
51  AATCAAAACC GAAGACATCA AACCCGCCCT GCAAACCGCC ATCGCCGAAG
101 CGCGCGAACA AATCGCCGCC ATCAAAGCCC AAACGCACAC CGGCTGGGCA
151 AACACTGTCTG AACCCCTGAC CGGCATCACC GAACGCGTCG GCAGGATTTG
201 GGGCGTGGTG TCGCACCTCA ACTCCGTCGC CGACACGCCC GAACTGCGCG
251 CCGTCTATAA CGAACTGATG CCCGAAATCA CCGTCTTCTT CACCGAAATC
301 GGACAAGACA TCGAGCTGTA CAACCGCTTC AAAACCATCA AAAATTCCCC
351 CGAATTGAC ACCTCTCTCC CCGCACAAAA AACCAAATC AACCACGATC
401 TGCGCGATTT CGTCTCAGC GCGCGGGAAC TGCCGCCCCG ACAGCAGGCA
451 GAATGGCAA AACTGCAAAC CGAAGGCGCG CAACTTTCG CCAAATCTC
501 CCAAACGTC CTAGACGCGA CCGACGCGTT CGGCATTAC TTTGACGATG
551 CCGCACCGCT TGCCGGCATT CCCGAAGACG CGCTCGCCAT GTTTGCCGCC
601 GCCGCGCAAA GCGAAAGCAA AACAGGCTAC AAAATCGGCT TGCAGATTCC
651 AACTACCTC GCCGTCTATC AATACGCCGA CAACCGCGAA CTGCGCGAAC
701 AAATCTACCG CGCCTACGTT ACCCGCGCQA GCGAACTTTC AGACGACGGC
751 AAATTCGACA ACACCGCCAA CATCGACCGC ACGCTCGCAA ACGCCCTGCA
801 AACCGCCAAA CTGCTCGGCT TCAAAAATA CGCCGAATTG TCGCTGGCAA
851 CCAAATGGC GGACACGCCC GAACAAGTTT TAACTTCTT GCACGACCTC
901 GCCGCGCGCG CCAAACCCTA CGCCGAAAAA GACCTCGCCG AAGTCAAAGC
951 CTTGCGCCGC GAAAGCCTGA ACCTCGCCGA TTTGCAACCG TGGGACTTGG
1001 GCTACGCCAG CGAAAAACTG CGCGAAGCCA AATACGCGTT CAGCGAAACC
1051 GAAGTCAAAA AATACTTCCC CGTCGGCAAA GTATTAAACG GACTGTTGCG
1101 CCAAATCAA AACTCTACG GCATCGGATT TACCGAAAAA ACCGTCCCCG
1151 TCTGGCACA AGACGTGCGC TATTTTGAAT TGCAACAAAA CGGCGAAACC
1201 ATAGGCGGCG TTTATATGGA TTTGTACGCA CGCGAAGGCA AACGCGGCGG
1251 CGCGTGGATG AACGACTACA AAGGCGCCG CCGTTTTTCA GACGCGACGC
1301 TGCAACTGCC CACCGCTAC CTCTGTCTGCA ACTTCGCCCC ACCCGTCGGC
1351 GGCAGGGAAG CCCGCCTGAG CCACGACGAA ATCCTCATCC TCTTCCACGA
1401 AACCGGACAC GGGCTGCACC ACCTGCTTAC CCAAGTGGAC GAACTGGGCG

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```

1451 TATCCGGCAT CAACGGCGTA GAATGGGACG CGGTGGAAC T GCGGAGCCAG
1501 TTTATGGAAA ATTTTCGTTT GGAATACAAT GTCTTGGCAC AAATGTCAGC
1551 CCACGAAGAA ACCGGCGTTC CCCTGCCGAA AGAACTCTTC GACAAAATGC
1601 TCGCCGCCAA AAACCTCCAA CGCGGCATGT TCCTCGTCCG GCAAATGGAG
1651 TTCGCCCTCT TTGATATGAT GATTACAGC GAAGACGACG AAGGCCGTCT
1701 GAAAAACTGG CAACAGGTTT TAGACAGCGT GCGCAAAAAA GTCGCCGTCA
1751 TCCAGCCGCC CGAATACAAC CGCTTCGCCT TGAGCTTCGG CCACATCTTC
1801 GCAGGCGGCT ATTCCGCAGG CTATTACAGC TACGCGTGGG CGGAAGTATT
1851 GAGCGCGGAC GCATACGCCG CCTTTGAAGA AAGCGACGAT GTCGCCGCCA
1901 CAGGCAAACG CTTTGGCAG GAAATCCTCG CCGTCGGCGG ATCGCGCAGC
1951 GCGGCAGAAT CCTTCAAAGC CTTCCGCGGC CGCGAACCGA GCATAGACGC
2001 ACTCTTGCGC CACAGCGGTT TCGACAACGC GGTCTGA

```

This corresponds to the amino acid sequence <SEQ ID 57; ORF 128-1>:

m128-1.pep.

```

1 MTDNALLHLG EEPRFDQIKT EDIKPALQTA IAEAREQIAA IKAQHTGWA
51 NTVEPLTGIT ERVGRWGVV SHLNSVADTP ELRAVYNELM PEITVFFTEI
101 GQDIELYNRF KTIKNSPEFD TLSPAQKTKL NHDLRDFVLS GAELPPEQQA
151 ELAKLQTEGA QLSAKFSQNV LDATDAFGIY FDDAAPLAGI PEDALAMFAA
201 AAQSESKTGY KIGLQIPHYL AVIQYADNRE LREQIYRAYV TRASELSDDG
251 KFDNTANIDR TLANALQTAK LLGFKNYAEL SLATKMADTP EQVLNFLHDL
301 ARRAKPYAEK DLAEVKAFAR ESLNLADLQP WDLGYASEKL REAKYAFSET
351 EVKKYFPVGK VLNGLFAQIK KLYGIGFTEK TVPVWHKDVR YFELQONGET
401 IGGVYMDLYA REGKRGGAWM NDYKGRRRFS DGTQLPLTAY LVCNFAPPVG
451 GREARLSHDE ILILFHETGH GLHHLLTQVD ELGVSGINGV EWDAVELEPSQ
501 FMENFVWEYN VLAQMSAHEE TGVPLPKELF DKMLAAKNFQ RGMFLVRQME
551 FALFDMMIYS EDDEGRLLKNW QQVLDVSRKK VAVIQPPEYN RFALSFGHIF
601 AGGYSAGYYS YAWAEVLSAD AYAAFEESDD VAATGKRFWQ EILAVGGSRS
651 AESFKAFRG REPSIDALLR HSGFDNAV*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 58>:

g128-1.seq (partial)

```

1 ATGATTGACA ACGCACTGCT CCACTTGGGC GAAGAACCCC GTTTTAATCA
51 AATCAAAACC GAAGACATCA AACC CGCGT CCAAACCGCC ATCGCCGAAG
101 CGCGCGGACA AATCGCCGCC GTCAAAGCGC AAACGCACAC CGGCTGGGCG
151 AACACCGTCG AGCGTCTGAC CGGCATCACC GAACGCGTCG GCAGGATTTG
201 GGGCGTCGTG TCCCATCTCA ACTCCGTCGT CGACACGCCC GAACTGCGCG
251 CCGTCTATAA CGAACTGATG CCTGAAATCA CCGTCTTCTT CACCGAAATC
301 GGACAAGACA TCGAACTGTA CAACCGCTTC AAAACCATCA AAAATTCCCC
351 CGAATTTGCA ACGCTTTCCC CCGCACAAAA AACCAAGCTC GATCACGACC
401 TGCGCGATT TCGTATTGAGC GCGCGGGAAC TGCGGCCCGA ACGCGAGGCA
451 GAACTGGCAA AACTGCAAC CGAAGGCGCG CAACTTTCCG CCAAAATCTC
501 CCAAAACGTC CTAGACGCGA CCGACGCGTT CGGCATTAC TTTGACGATG
551 CCGCACCGCT TGCCGGCATT CCCGAAGACG CGCTCGCCAT GTTGCCCGCC
601 GCGCGCAAAA GCGAAGGCAA AACAGGTTAC AAAATCGGCT TGCAGATTCC
651 GCACTACCTT GCGGTTATCC AATACGCGCG CAACCGCGAA CTGCGCGAAC
701 AAATCTACCG CGCTACGTT ACCCGTGCCA GCGAACTTTC AAACGACGGC
751 AAATTCGACA ACACCGCCAA CATCGACCGC ACGCTCGAAA ACGCATTGAA
801 AACCGCCAAA CTGCTCGGCT TAAAAAATTA CGCCGAATTG TCGCTGGCAA
851 CCAAATGGC GGACACGCCC GAACAGGTTT TAAACTTCCT GCACGACCTC
901 GCGCGCGCG CCAAACCTTA CGCCGAAAAA GACCTCGCCG AAGTCAAAGC
951 CTTGCGCCGC GAACACCTCG GTCTCGCCGA CCGCGAGCCG TGGGACTTGA
1001 GCTACGCGCG CGAAAACTG CGCGAAGCCA AATACGCATT CAGCGAAACC
1051 GAAGTCAAAA AATACTTCCC CGTCGGCAAA GTTCTGGCAG GCCTGTTCCG
1101 CCAAATCAAA AAATCTACG GCATCGGATT CGCCGAAAAA ACCGTTCCCG
1151 TCTGGCACAA AGACGTGCGC TATTTTGAAT TGCAACAAAA CGGCAAACCC
1201 ATCGGCGCGC TTTATATGGA TTTGTACGCA CGCGAAGGCA AACCGCGCGG
1251 CGCGTGGATG AACGACTACA AAGGCCGCCG CCGCTTTGCC GACGGCACGC
1301 TGCAACTGCC CACCGCCTAC CTGCTCTGCA ACTTCGCCCC GCGGTCGGC
1351 GGCAAGAAG CGGTTTAAAG CCACGACGAA ATCCTCACCC TCTTCCACGA
1401 AACCGGCCAC GGAATGACAC ACCTGCTTAC CCAAGTGGAC GAACTGGGCG
1451 TGTCGGGCAT CAACGGCGTA AAA

```

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This corresponds to the amino acid sequence <SEQ ID 59; ORF 128-1.ng>:

g128-1.pep (partial)

```

1  MIDNALLHLG EEPRFNQIKT EDIKPAVQTA IAEARGQIAA VKAQTHTGWA
51  NTVERTLGIT ERVGRIWGVV SHLNSVVDTP ELRAVYNELM PEITVFFTEI
101 GQDIELYNRF KTIKNSPEFA TLSPAQKTKL DHDLRDFVLS GAELPPERQA
151 ELAKLQTEGA QLSAKFSQNV LDATDAFGIY FDDAAPLAGI PEDALAMFAA
201 AAQSEGKTGY KIGLQIPHYL AVIQYAGNRE LREQIYRAYV TRASELSNDG
251 KFDNTANIDR TLENALKTAK LLGFKNYAEL SLATKMADTP EQVLNFLHDL
301 ARRAKPYAEK DLAEVKAFAR EHLGLADPQP WDLSYAGEKL REAKYAFSET
351 EVKKYFPVGK VLAGLFAQIK KLYGIGFAEK TVPVWHKDVR YFELQONGKT
401 IGGVYMDLYA REGKRGGAWM NDYKGRRRFA DGTQLQLPTAY LVCNFAPPVG
451 GKEARLSHDE ILTLFHETGH GLHLLTQVD ELGVSGINGV K

```

m128-1/g128-1 ORFs 128-1 and 128-1.ng showed a 94.5% identity in 491 aa overlap

g128-1.pep	10	20	30	40	50	60
	MIDNALLHLGEEPRFNQIKTEDIKPAVQTAIAEARGQIAAVKAQTHTGWANTVERLTGIT					
m128-1	MTDNALLHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAAIKAQTHTGWANTVEPLTGIT					
	10	20	30	40	50	60
g128-1.pep	70	80	90	100	110	120
	ERVGRIWGVVSHLNSVVDTPELRAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFA					
m128-1	ERVGRIWGVVSHLNSVADTPELRAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD					
	70	80	90	100	110	120
g128-1.pep	130	140	150	160	170	180
	TLSPAQKTKLDHDLRDFVLSGAELPPERQAELAKLQTEGAQLSAKFSQNVLDATDAFGIY					
m128-1	TLSPAQKTKLNHDLRDFVLSGAELPPEQQAELAKLQTEGAQLSAKFSQNVLDATDAFGIY					
	130	140	150	160	170	180
g128-1.pep	190	200	210	220	230	240
	FDDAAPLAGIPEDALAMFAAAQSEGKTGYKIGLQIPHYLAVIQYAGNRELREQIYRAYV					
m128-1	FDDAAPLAGIPEDALAMFAAAQSESKTGYKIGLQIPHYLAVIQYADNRELREQIYRAYV					
	190	200	210	220	230	240
g128-1.pep	250	260	270	280	290	300
	TRASELSNDGKFDNTANIDRTLENALKTAKLLGFKNYAELSLATKMADTPEQVLNFLHDL					
m128-1	TRASELSDDGKFDNTANIDRTLANALQTAKLLGFKNYAELSLATKMADTPEQVLNFLHDL					
	250	260	270	280	290	300
g128-1.pep	310	320	330	340	350	360
	ARRAKPYAEKDLAEVKAFAREHLGLADPQPWDLSYAGEKLREAKYAFSETEVKKYFPVGK					
m128-1	ARRAKPYAEKDLAEVKAFARESLNLADLPWDLGYASEKLREAKYAFSETEVKKYFPVGK					
	310	320	330	340	350	360
g128-1.pep	370	380	390	400	410	420
	VLAGLFAQIKKLYGIGFAEKTVPVWHKDVR YFELQONGKTIGGVYMDLYAREGKRGGAWM					
m128-1	VLNGLFAQIKKLYGIGFTEKTVPVWHKDVR YFELQONGKTIGGVYMDLYAREGKRGGAWM					
	370	380	390	400	410	420
g128-1.pep	430	440	450	460	470	480
	NDYKGRRRFADGTQLQLPTAYLVCNFAPPVGGKEARLSHDEILTLFHETGHGLHLLTQVD					

```

|||||||:|||||||:|||||||
m128-1 NDYKGRRRFSDGTLQLPTAYLVCNFAPPVGGREARLSHDEILILFHETGHGLHHLTQVD
          430          440          450          460          470          480

          490
g128-1.pep ELGVSGINGVK
          |||||:
m128-1 ELGVSGINGVEWDAVELPSQFMENFVWEYNVLAQMSAHEETGVPLPKELFDKMLAAKNFQ
          490          500          510          520          530          540

```

**a128-1.seq**

This corresponds to the amino acid sequence <SEQ ID 61; ORF 128-1.a>:

a128-1.pep

1	MTDNALLHLG	EEPRFDQIKT	EDIKPALQTA	IAEAREQIAA	IKAQTHGWA
51	NTVEPLTGIT	ERVGRIWGVV	SHLSVTDTP	ELRAAYNELM	PEITVFFTEI
101	GQDIELYNRF	KTIKNSPEFD	TLSHAQTKKL	NHDLRDFVLS	GAELPPEQQA
151	ELAKLQSGTA	QLSAKFSQNV	LDATDAFGIY	FDDAAPLAGI	PEDALAMFAA
201	AAQSEKGTGY	KIGLQIPHYL	AVIQYADNRK	LREQIYRAYV	TRASELSDDG
251	KFDNTANIDR	TLENALQTAK	LLGFKNYAEL	SLATKMADTP	EOVLNLFHDL

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```

301  ARRAKPYA EKDLAEVKAFAR  ESLGLADLQP  WDLGYAGEKL  REAKYAFSET
351  EVKKYFPV GKVLNGLFAQIK  KLYGIGFTEK TVPVWHKDVR  YFELQONGET
401  IGGVYMDLYA REGKRGGA WMNDYKGRRRFS  DGTLLQLPTAY LVCNFTPPVG
451  GKEARLSHDE ILTLFHETGH  GLHLLLTQVD  ELGVSGINGV  EWD AVELPSQ
501  FMENFVWEYN VLAQMSAHEE  TGVPLPKELF  DKMLAAKNFQ  RGMFLVRQME
551  FALFDMMIYS EDDEGR LKNWQQVLDSVRKE  VAVVRPPEYN  RFANSFGHIF
601  AGGYSAGYYS YAWAEVLSAD  AYA AFEESDD  VAATGKRFWQ  EILAVGGSRS
651  AAESFKAFRG REPSIDALLR  HSGFDNAA*

```

**m128-1/a128-1** ORFs 128-1 and 128-1.a showed a 97.8% identity in 677 aa overlap

	10	20	30	40	50	60
a128-1.pep	MTDNALLHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAAIIKAQTHGTWANTVEPLTGIT					
m128-1	MTDNALLHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAAIIKAQTHGTWANTVEPLTGIT					
	10	20	30	40	50	60
	70	80	90	100	110	120
a128-1.pep	ERVGRIGWVSHLNSVDTPELRAAYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD					
m128-1	ERVGRIGWVSHLNSVADTPELRAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD					
	70	80	90	100	110	120
	130	140	150	160	170	180
a128-1.pep	TLSHAQKTKLNHDLRDFVLSGAELPPEQQAELAKLQTEGAQLSAKFSQNVLDATDAFGIY					
m128-1	TLSPAQKTKLNHDLRDFVLSGAELPPEQQAELAKLQTEGAQLSAKFSQNVLDATDAFGIY					
	130	140	150	160	170	180
	190	200	210	220	230	240
a128-1.pep	FDDAAPLAGIPEDALAMFAAAQSEKGTGYKIGLQIPHYLAVIQYADNRKLRQIYRAYV					
m128-1	FDDAAPLAGIPEDALAMFAAAQSEKGTGYKIGLQIPHYLAVIQYADNRKLRQIYRAYV					
	190	200	210	220	230	240
	250	260	270	280	290	300
a128-1.pep	TRASELSDDGKFDNTANIDRTLENALQTAKLLGFKNYAELSLATKMDTPEQVLNFLHDL					
m128-1	TRASELSDDGKFDNTANIDRTLANALQTAKLLGFKNYAELSLATKMDTPEQVLNFLHDL					
	250	260	270	280	290	300
	310	320	330	340	350	360
a128-1.pep	ARRAKPYAEKDLAEVKAFARESLGLADLQPWDLGYAGEKLREAKYAFSETEVKKYFPV GK					
m128-1	ARRAKPYAEKDLAEVKAFARESLNLADLQPWDLGYASEKLREAKYAFSETEVKKYFPV GK					
	310	320	330	340	350	360
	370	380	390	400	410	420
a128-1.pep	VLNGLFAQIKKLYGIGFTEKTPVWHKDVRYFELQONGETIGGVYMDLYAREGKRGGA WM					
m128-1	VLNGLFAQIKKLYGIGFTEKTPVWHKDVRYFELQONGETIGGVYMDLYAREGKRGGA WM					
	370	380	390	400	410	420
	430	440	450	460	470	480
a128-1.pep	NDYKGRRRFS DGTLLQLPTAYLVCNFTPPVGGKEARLSHDEILTLFHETGHGLHLLTQVD					
m128-1	NDYKGRRRFS DGTLLQLPTAYLVCNFAPPVGGREARLSHDEILILFHETGHGLHLLTQVD					
	430	440	450	460	470	480
	490	500	510	520	530	540
a128-1.pep	ELGVSGINGVEWDAVELPSQFMENFVWEYNVLAQMSAHEETGVPLPKELFDKMLAAKNFQ					

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```

m128-1      ELGVSGINGVEWDAVELPSQFMENFVWEYNVLAQMSAHEETGVPLPKELFDKMLAAKNFQ
              490      500      510      520      530      540

              550      560      570      580      590      600
a128-1.pep  RGMFLVRQMEFALFDMMIYSEDDEGRKKNWQQVLDVRKEVAVVRPPEYNRFANSFGHIF
              |||||:||||:|||||
m128-1      RGMFLVRQMEFALFDMMIYSEDDEGRKKNWQQVLDVRKKVAVIQPPEYNRFALSFGHIF
              550      560      570      580      590      600

              610      620      630      640      650      660
a128-1.pep  AGGYSAGYYSYAWAEVLSADAYAAFEESDDVAATGKRFWQEILAVGGSRSAAESFKAFRG
              |||||:||||:|||||
m128-1      AGGYSAGYYSYAWAEVLSADAYAAFEESDDVAATGKRFWQEILAVGGSRSAAESFKAFRG
              610      620      630      640      650      660

              670      679
a128-1.pep  REPSIDALLRHSGFDNAAX
              |||||:
m128-1      REPSIDALLRHSGFDNAVX
              670

```

206

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 62>:

```

m206.seq
1   ATGTTTCCCC CCGACAAAAC CCTTTTCCTC TGTCTCAGCG CACTGCTCCT
51  CGCCTCATGC GGCACGACCT CCGGCAAACA CCGCCAACCG AAACCCAAAC
101 AGACAGTCCG GCAAATCCAA GCCGTCCGCA TCAGCCACAT CGACCGCACA
151 CAAGGCTCGC AGGAATCAT  GCTCCACAGC CTCGGACTCA TCGGCACGCC
201 CTACAAATGG GCGGCAGCA  GCACCGCAAC CGGCTTCGAT TGCAGCGGCA
251 TGATTCAATT CGTTTACAAr AACGCCCTCA ACGTCAAGCT GCCGCGCACC
301 GCGCGCGACA TGGCGGCGGC AAGCCGsAAA ATCCCGGAcA GCCGcYTCaa
351 GGCGGCGGAC CTCGTATTCT TCAACACCGG CGGCGCACAC CGCTACTCAC
401 ACGTCGGACT CTACATCGGC AACGGCGAAT TCATCCATGC CCCCAGCAGC
451 GGCAAAACCA TCAAAACCGA AAAACTCTCC ACACCGTTTT ACGCCAAAAA
501 CTACCTCGGC GCACATACTT TTTTACAGA ATGA

```

This corresponds to the amino acid sequence <SEQ ID 63; ORF 206>:

```

m206.pep..
1   MFPPDKTLFL CLSALLLASC GTTSGKHRQP KPKQTVRQIQ AVRISHIDRT
51  QGSQELMLHS LGLIGTPYKW GGSSTATGFD CSGMIQFVYK NALNVKLPRT
101 ARDMAAASRK IPDSRXKAGD LVFFNTGGAH RYSHVGLYIG NGEFIHAPSS
151 GKTIKTEKLS TPFYAKNYLG AHTFFTE*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 64>:

```

g206.seq
1   atgttttccc ccgacaaaac ccttttcctc tgtctcggcg cactgctcct
51  cgcctcatgc ggcacgacct ccggcaaaca ccgccaacgg aaacccaaac
101 agacagtccg gcaaattcaa gccgtccgca tcagccacat cggcgcgaca
151 caaggctcgc aggaactcat gctccacagc ctccggactca tcggcacgcc
201 ctacaaatgg ggcggcgagc gcaccgcaac cggcttcgac tgcagcggca
251 tgattcaatt ggtttacaaa aacgccctca acgtcaagct gccgcgcacc
301 gcccgcgaca tggcggcggc aagccgcaaa atccccgaca gccgcctcaa
351 ggccggcgac atcgtattct tcaacaccgg cggcgcacac cgctactcac
401 acgtcggact ctacatcggc aacggcgaat tcattccatgc ccccggcgagc
451 ggcaaaacca tcaaaaccga aaaactctcc acaccgtttt acgccaacaaa
501 ctaccttgga gcgcatacgt tttttacaga atga

```

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This corresponds to the amino acid sequence <SEQ ID 65; ORF 206.ng>:

```

g206.pep
  1  MFSPDKTLFL CLGALLLASC GTTSGKHRQP KPKQTVRQIQ AVRISHIGRT
 51  QGSQELMLHS LGLIGTPYKW GGSSTATGFD CSGMIQLVYK NALNVKLPR
101  ARDMAAASRK IPDSRLKAGD IVFFNTGGAH RYSHVGLYIG NGEFIHAPGS
151  GKTIKTEKLS TPFYAKNYLG AHTFFTE*

```

ORF 206 shows 96.0% identity over a 177 aa overlap with a predicted ORF (ORF 206.ng) from *N. gonorrhoeae*:

```

m206/g206

      10      20      30      40      50      60
m206.pep  MFPPDKTLFLCLSALLLASC GTTSGKHRQPKPKQTVRQIQAVRISHIDRTQGSQELMLHS
          |||||||:|||||
g206       MFSPDKTLFLCLGALLLASC GTTSGKHRQPKPKQTVRQIQAVRISHIGRTQGSQELMLHS
          10      20      30      40      50      60

      70      80      90     100     110     120
m206.pep  LGLIGTPYKWGGSSTATGFD CSGMIQFVYKNALNVKLPR TARDMAAASRKIPDSRXKAGD
          |||||||:|||||
g206       LGLIGTPYKWGGSSTATGFD CSGMIQLVYKNALNVKLPR TARDMAAASRKIPDSRLKAGD
          70      80      90     100     110     120

      130     140     150     160     170
m206.pep  LVFFNTGGAHRYSHVGLYIGNGEFIHAPSSGKTIKTEKLSTPFYAKNYLGAHTFFTEX
          :|||||:|||||
g206       IVFFNTGGAHRYSHVGLYIGNGEFIHAPSGKTIKTEKLSTPFYAKNYLGAHTFFTE
          130     140     150     160     170

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 66>:

```

a206.seq
  1  ATGTTTCCCC CCGACAAAAC CCTTTTCCTC TGTCTCAGCG CACTGCTCCT
 51  CGCCTCATGC GGCACGACCT CCGGCAAACA CCGCCAACCG AAACCCAAAC
101  AGACAGTCCG GCAAATCCAA GCCGTCCGCA TCAGCCACAT CGACCGCACA
151  CAAGGCTCGC AGGAACTCAT GCTCCACAGC CTCGGACTCA TCGGCACGCC
201  CTACAAATGG GCGGCAGCA GCACCGCAAC CGGCTTCGAT TGCAGCGGCA
251  TGATTCAATT CGTTTACAAA AACGCCCTCA ACGTCAAGCT GCCGCGCACC
301  GCCCGCGACA TGGCGGCGGC AAGCCGCAAA ATCCCGGACA GCCGCCTTAA
351  GGCCGGCGAC CTCGTATTCT TCAACACCGG CGGCGCACAC CGCTACTCAC
401  ACGTCGGACT CTATATCGGC AACGGCGAAT TCATCCATGC CCCCAGCAGC
451  GGCAAAACCA TCAAAACCGA AAAACTCTCC ACACCGTTT ACGCCAAAAA
501  CTACCTCGGC GCACATACTT TCTTTACAGA ATGA

```

This corresponds to the amino acid sequence <SEQ ID 67; ORF 206.a>:

```

a206.pep
  1  MFPPDKTLFL CLSALLLASC GTTSGKHRQP KPKQTVRQIQ AVRISHIDRT
 51  QGSQELMLHS LGLIGTPYKW GGSSTATGFD CSGMIQFVYK NALNVKLPR
101  ARDMAAASRK IPDSRLKAGD LVFFNTGGAH RYSHVGLYIG NGEFIHAPSS
151  GKTIKTEKLS TPFYAKNYLG AHTFFTE*

```

m206/a206 ORFs 206 and 206.a showed a 99.4% identity in 177 aa overlap

```

      10      20      30      40      50      60
m206.pep  MFPPDKTLFLCLSALLLASC GTTSGKHRQPKPKQTVRQIQAVRISHIDRTQGSQELMLHS
          |||||||
a206       MFPPDKTLFLCLSALLLASC GTTSGKHRQPKPKQTVRQIQAVRISHIDRTQGSQELMLHS
          10      20      30      40      50      60

```

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	70	80	90	100	110	120
m206.pep	LGLIGTPYKWGGSSTATGFDCSGMIQFVYKNALNVKLPRTARDMAAASRKIPDSRXKAGD					
a206	LGLIGTPYKWGGSSTATGFDCSGMIQFVYKNALNVKLPRTARDMAAASRKIPDSRLKAGD					
	70	80	90	100	110	120
	130	140	150	160	170	
m206.pep	LVFFNTGGAHRYSHVGLYIGNGEFIHAPSSGKTIKTEKLSTPFYAKNYLGAHTFFTEX					
a206	LVFFNTGGAHRYSHVGLYIGNGEFIHAPSSGKTIKTEKLSTPFYAKNYLGAHTFFTEX					
	130	140	150	160	170	

287

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 68>:

```

m287.seq
1  ATGTTTAAAC GCAGCGTAAT CGCAATGGCT TGTATTTTTG CCCTTTCAGC
51  CTGCGGGGGC GCGGGTGGCG GATCGCCCGA TGTCAAGTCG GCGGACACGC
101 TGTCAAACC TGCGCCCTT GTTGTCTCTG AAAAAGAGAC AGAGGCAAAG
151 GAAGATGCGC CACAGGCAGG TTCTCAAGGA CAGGGCGCGC CATCCGCACA
201 AGGCAGTCAA GATATGGCGG CGGTTTCGGA AGAAAATACA GGCAATGGCG
251 GTGCGGTAAC AGCGGATAAT CCCAAAAATG AAGACGAGGT GGCACAAAAT
301 GATATGCCGC AAAATGCCGC CGGTACAGAT AGTTCGACAC CGAATCACAC
351 CCCGGATCCG AATATGCTTG CCGGAAATAT GGAATCAAA GCAACGGATG
401 CCGGGGAATC GTCTCAGCGG GCAAACCAAC CGGATATGGC AAATGCGGCG
451 GACGGAATGC AGGGGGACGA TCCGTCGGCA GCGGGGCAA ATGCGGGCAA
501 TACGGCTGCC CAAGGTGCAA ATCAAGCCGG AAACAATCAA GCCGCCGGTT
551 CTTAGATCC CATCCCCGCG TCAAACCTTG CACCTGCGAA TGGCGGTAGC
601 AATTTGGAA GGGTTGATTT GGCTAATGGC GTTTTGATTG ACGGGCCGTC
651 GCAAAATATA ACGTTGACCC ACTGTAAAGG CGATTCTTGT AGTGCCAATA
701 ATTTCTTGA TGAAGAAGTA CAGCTAAAT CAGAATTGA AAAATTAAGT
751 GATGAGACA AAATAAGTAA TTACAAGAAA GATGGGAAGA ATGATAAATT
801 TGTCGGTTTG GTTGCCGATA GTGTGCAGAT GAAGGGAATC AATCAATATA
851 TTATCTTTA TAAACCTAAA CCCACTCAT TTGCGCGATT TAGGCGTTCT
901 GCACGGTCGA GCGGTCGCT TCCGCGCGAG ATGCCGCTGA TTCCCGTCAA
951 TCAGGCGGAT ACGCTGATTG TCGATGGGGA AGCGGTCAGC CTGACGGGGC
1001 ATTCCGGCAA TATCTTCGCG CCCGAAGGGA ATTACCGGTA TCTGACTTAC
1051 GGGGCGGAAA AATTGCCCCG CGGATCGTAT GCCCTTCGTG TTCAAGGCGA
1101 ACCGGCAAAA GCGGAAATGC TTGCGGGCGC GGCCGTGTAC AACGGCGAAG
1151 TACTGCATTT CCATACGGAA AACGGCCGTC CGTACCCGAC CAGGGGAGG
1201 TTTGCCGCAA AAGTCGATTT CGGCAGCAA TCTGTGGACG GCATTATCGA
1251 CAGCGCGCAT GATTTGCATA TGGGTACGCA AAAATTCAAA GCCGCCATCG
1301 ATGGAAACGG CTTTAAGGGG ACTTGGACGG AAAATGGCAG CGGGGATGTT
1351 TCCGAAAGT TTTACGGCCC GGCCGGCGAG GAAGTGGCGG GAAAATACAG
1401 CTATCGCCCG ACAGATGCGG AAAAGGGCGG ATTCGGCGTG TTTGCCGCGA
1451 AAAAAGAGCA GGATTGA

```

This corresponds to the amino acid sequence <SEQ ID 69; ORF 287>:

```

m287.pep
1  MFKRSVIAMA CIFALSACGG GGGGSPDVKS ADTLKPAAP VVSEKETEAQ
51  EDAPQAGSQ QGAPSAQGSQ DMAAVSEENT GNGGAVTADN PKNEDEVAQN
101 DMPQNAAGTD SSTPNHTPDP NMLAGNMENQ ATDAGESSQP ANQPDMAAAA
151 DGMQDDPSA GGQNAAGTAA QGANQAGNNQ AAGSSDPIPA SNPAPANGGS
201 NFGFVDLANG VLIDGPSQNI TLTHCKGDSC SGNNFLDEEV QLKSEFEKLS
251 DADKISNYKK DGKNDKFVGL VADSVQMKGI NQYIIFYKPK PTSFARFRRS
301 ARSRRSLPAE MPLIPVNQAD TLIVDGEAVS LTGHSGNIFA PEGNYRYLTY

```

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351 GA EKLP GGSY ALRVQGEPAK GEMLAGAAVY NGEVLHFHTE NGRPYPTRGR  
 401 FAAKVD FGSK SVDGIIDSGD DLHMG TQKFK AAIDGN GFKG TWTENGSGDV  
 451 SGKFYGPAGE EVAGKYSYRP TDAEKG GFGV FAGKKEQD\*

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 70>:

g287.seq  
 1 atgtttaaac gcagtgtgat tgcaatggct tgtatttttc ccctttcagc  
 51 ctgtgggggc ggcggtggcg gatcgcccga tgtcaagtcg gcggacacgc  
 101 cgtcaaaacc ggccgcccc gttgttgctg aaaatgccgg ggaagggtg  
 151 ctgccgaaag aaaagaaaga tgaggaggca gcgggcggtg cgccgcaagc  
 201 cgatacgagc gacgcaaccg ccggagaagg cagccaagat atggcggcag  
 251 tttcggcaga aaatacaggc aatggcggtg cgcaacaac ggacaacccc  
 301 aaaaatgaag acgcgggggc gcaaaatgat atgccgcaaa atgccgcca  
 351 atccgcaaat caaacaggga acaaccaacc cgccggttct tcagattccg  
 401 cccccgcgtc aaaccctgcc cctgcgaatg gcggtagcga ttttggaagg  
 451 acgaacgtgg gcaattctgt tgtgattgac ggaccgtcgc aaaaataaac  
 501 gttgaccac tgtaaaggcg attcttgtaa tggtgataat ttattggatg  
 551 aagaagcacc gtcaaaatca gaatttga aaattaagtga tgaagaaaaa  
 601 attaaagcgt ataaaaaaga cgagcaacgg gagaattttg tcggtttggt  
 651 tgctgacagg gtaaaaaagg atggaactaa caaatatata atcttctata  
 701 cggacaaaac acctactcgt tctgcacggt cgaggaggtc gcttccggcc  
 751 gagattccgc tgattcccgt caatcaggcc gatacgctga ttgtggatgg  
 801 ggaagcgtc agcctgacgg ggcattccgg caatatcttc gcgcccgaag  
 851 ggaattaccg gtatctgact tacggggcgg aaaaattgcc cgcgcgatcg  
 901 tatgccctcc gtgtgcaagg cgaaccggca aaaggcgaaa tgcttgttgg  
 951 cacggccgtg tacaacggcg aagtgtgca tttccatatg gaaaacggcc  
 1001 gtccgtaccc gtccggaggc aggtttgccg caaaagtcga tttcggcagc  
 1051 aaatctgtgg acggcattat cgacagcggc gatgatttgc atatgggtac  
 1101 gcaaaaattc aaagccgcca tcgatgaaa cggtttaag gggacttgga  
 1151 cggaaaatgg cggcggggat gtttccggaa ggttttacgg cccggccggc  
 1201 gaggaagtgg cgggaaaata cagctatcgc ccgacagatg ctgaaaaggg  
 1251 cggattcggc gtgtttgccg gcaaaaaaga tcgggattga

This corresponds to the amino acid sequence <SEQ ID 71; ORF 287.ng>:

g287.pep  
 1 MFKRSVIAMA CIFPLSACGG GGGGSPDVKS ADTPSKPAAP VVAENAGEGV  
 51 LPKEKKDEEA AGGAPQADTQ DATAGEGSQD MAAVSAENTG NGGAATTDNP  
 101 KNEDAGA QND MPQNAAESAN QTGNQAPGS SDSAPASNPA PANGGSDFR  
 151 TNVGNSSVID GPSQNTLTH CKGDSCNGDN LLDEEAPSKS EFEKLSDEEK  
 201 IKRYKKDEQR ENFVGLVADR VKKDGTNKYI IFYTDKPPTR SARSRRSLPA  
 251 EIPVIPVQA DTLLVDGEAV SLTGHSGNIF APEGNYRYLT YGA EKLP GGS  
 301 YALRVQGEPA KGEMLVGTAV YNGEVLHFHM ENGRPYPSGG RFAAKVD FGS  
 351 KSVDGIIDSG DDLHMG TQKFK KAAIDGN GFK GTWTENG GGD VSGRFYGPAG  
 401 EEVAGKYSYR PTDAEKG GFG VFAGKKDRD\*

m287/g287 ORFs 287 and 287.ng showed a 70.1% identity in 499 aa overlap

	10	20	30	40	49
m287.pep	MFKRSVIAMACIFALSACGGGGGGSPDVKSADT	LSKPAAPVVSE	-----KETEA		
g287	MFKRSVIAMACIFPLSACGGGGGGSPDVKSADT	PSKPAAPVVAENAGEGVLPKEKKDEEA			
	10	20	30	40	50
	50	60	70	80	90
m287.pep	KEDAPQAGSQGQAPS	AQGSQDMAAVSEENTGNGGAVTADNPKNEDEVAQNDMPQNAAGT	100	109	
g287	AGGAPQADTQD--	ATAGEGSQDMAAVSAENTGNGGAATTDNPKNEDAGAQN	110		



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m287.pep	110	120	130	140	150	160	169
	DSSTPNHTPDFNMLAGNMENQATDAGES SQPANQPDMANAADGMQGDPSAGGQNAGNTA						
g287	-----						
m287.pep	170	180	190	200	210	220	229
	AQGANQAGNNQAAGSSDPIPASNPAPANGGSNFRVLDLANGVLIDGPSQNTLTHCKGDS						
g287	-ESANQTGNNQPAGSSDSAPASNPAPANGGSDFGRTNVGNSVVIDGPSQNTLTHCKGDS						
	120	130	140	150	160	170	
m287.pep	230	240	250	260	270	280	289
	CSGNNFLDEEVQLKSEFEKLSADAKISNYKKDGKNDKFVGLVADSVQMKGINQYIIIFYKP						
g287	CNGDNLDEEAPSKSEFEKLSDEEKIKRYKKDEQRENFVGLVADRVKKDGTNKYIIIFYTD						
	180	190	200	210	220	230	
m287.pep	290	300	310	320	330	340	349
	KPTSFAFRFRSARSRRSLPAEMPLIPVNQADTLIVDGEAVSLTGHSGNIFAPEGNYRYLT						
g287	KPPT-----RSARSRRSLPAEIPVNQADTLIVDGEAVSLTGHSGNIFAPEGNYRYLT						
	240	250	260	270	280	290	
m287.pep	350	360	370	380	390	400	409
	YGAEKLPGGSYALRVQGEPAKGEMLAGAAVYNGEVLHFHTENGRPYPTRGRFAAKVDFGS						
g287	YGAEKLPGGSYALRVQGEPAKGEMLVGTAVYNGEVLHFHMENGRPYPSGGRFAAKVDFGS						
	300	310	320	330	340	350	
m287.pep	410	420	430	440	450	460	469
	KSVDGIIDSGDDLHMGTKQFKAAIDGNGFKGTWTENGSGDVSGKFYGPAGEEVAGKYSYR						
g287	KSVDGIIDSGDDLHMGTKQFKAAIDGNGFKGTWTENGSGDVSGRFYGPAGEEVAGKYSYR						
	360	370	380	390	400	410	
m287.pep	470	480	489				
	PTDAEKGFGVFAGKKEQDX						
g287	PTDAEKGFGVFAGKKDRDX						
	420	430					

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 72>:

```

a287.seq
1  ATGTTTAAAC GCAGTGTGAT TGCAATGGCT TGTATTGTTG CCCTTTCAGC
51 CTGTGGGGGC GCGGTGGCG GATCGCCGA TGTAAAGTCG GCGGACACGC
101 TGTCAAACC TGCCGCCCT GTTGTTACTG AAGATGTCGG GGAAGAGGTG
151 CTGCCGAAAG AAAAGAAAGA TGAGGAGGCG GTGAGTGGTG CGCCGCAAGC
201 CGATACGCAG GACGCAACCG CCGGAAAAGG CGGTCAAGAT ATGGCGGCAG
251 TTTCGGCAGA AAATACAGGC AATGGCGGTG CGGCAACAAC GGATAATCCC
301 GAAATAAAG ACGAGGGACC GCAAATGAT ATGCCGCAA ATGCCGCCGA
351 TACAGATAGT TCGACACCGA ATCACACCCC TGCACCGAAT ATGCCAACCA
401 GAGATATGGG AAACCAAGCA CCGGATGCGG GGAATCGGC ACAACCGCA
451 AACCAACCGG ATATGGCAA TGCGGCGGAC GGAATGCAGG GGGACGATCC
501 GTCGGCAGG GAAATGCCG GCAATACGGC AGATCAAGCT GCAAATCAAG
551 CTGAAAACAA TCAAGTCGGC GGCTCTCAA ATCCTGCCTC TTCAACCAAT
601 CCTAACGCCA CGAATGGCG CAGCGATTTT GGAAGGATAA ATGTAGCTAA
651 TGGCATCAAG CTTGACAGCG GTTCGAAAA TGTAACGTTG ACACATTGTA
701 AAGACAAAGT ATGCGATAGA GATTCTTAG ATGAAGAAGC ACCACCAAAA
751 TCAGAAATTG AAAAAATTAAG TGATGAAGAA AAAATTAATA AATATAAAAA
801 AGACGAGCAA CGAGAGAATT TTGTCGGTTT GGTGCTGAC AGGGTAGAAA

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851 AGAATGGAAC TAACAAATAT GTCATCATTT ATAAAGACAA GTCCGCTTCA
901 TCTTCATCTG CGCGATTTCAG GCGTTCTGCA CGGTCGAGGC GGTGCGTTCC
951 GGCCGAGATG CCGCTGATTC CCGTCAATCA GCGCGATACG CTGATTGTCTG
1001 ATGGGGAAGC GGTCAGCCTG ACGGGGCATT CCGGCAATAT CTTCCGCGCC
1051 GAAGGGAATT ACCCGGTATCT GACTTACGGG GCGGAAAAAT TGTCCGGCCG
1101 ATCGTATGCC CTCAGTGTGC AAGGCGAACC GGCAAAAGGC GAAATGCTTG
1151 CGGGCACGGC CGTGTACAAC GCGGAAGTGC TGCATTTCCA TATGGAAAAC
1201 GGCCGTCCGT CCCCCTCCGG AGGCAGGTTT GCCGAAAAG TCGATTTCCG
1251 CAGCAAAATCT GTGGACGGCA TTATCGACAG CCGCGATGAT TTGCATATGG
1301 GTACGCAAAA ATTCAAAGCC GTTATCGATG GAAACGGCTT TAAGGGGACT
1351 TGGACGAAA ATGGCGGCGG GGATGTTTCC GGAAGGTTT ACGGCCCGGC
1401 CGGCGAAGAA GTGGCGGGA AATACAGCTA TCGCCCGACA GATGCGGAAA
1451 AGGGCGGATT CCGCGTGT TTGCCGCAAAA AAGAGCAGGA TTGA

```

This corresponds to the amino acid sequence <SEQ ID 73; ORF 287.a>:

a287.pep

```

1 MFKRSVIAMA CIVALSAACGG GGGGSPDVKS ADTLSKPAAP VVTEDVGEEV
51 LPKEKKDEEA VSGAPQADTQ DATAGKGGQD MAAVSAENTG NGGAATTNDP
101 ENKDEGPOND MPQNAADTDS STPNHTPAPN MPTRDMGNQA PDAGESAQPA
151 NQPDMANAAD GMQDDPSAG ENAGNTADQA ANQAENNQVG GSONPASSTN
201 PNATNGGSDF GRINVANGIK LDSGSENVTL THCKDKVCDR DFLDEEAPPK
251 SEFEKLSDEE KINKYKKDEQ RENFVGLVAD RVEKNGTNKY VIIYKDKSAS
301 SSSARFRRSA RSRRLPAEM PLIPVQADT LIVDGEAVSL TGHSGNIFAP
351 EGNRYLYTG AEKLSGGSYA LSVQGEPAKG EMLAGTAVYN GEVLHFHMEN
401 GRPSPSGGRF AAKVDFGSKS VDGIIIDSGDD LHMGTQKFKA VIDNGFGKGT
451 WTENGGGDVS GRFYGPAGEE VAGKYSYRPT DAEKGGFGVF AGKKEQD*

```

m287/a287 ORFs 287 and 287.a showed a 77.2% identity in 501 aa overlap

```

              10      20      30      40      49
m287.pep      MFKRSVIAMACIFALSACGGGGGGSPDVKSADTLSKPAAPVVSE-----KETEA
              ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
a287          MFKRSVIAMACIVALSAACGGGGGGSPDVKSADTLSKPAAPVVTEDEVGEEVLPKEKKDEEA
              10      20      30      40      50      60

              50      60      70      80      90      100      109
m287.pep      KEDAPQAGSQGQAPSAGSODMAAVSEENTGNGGAVTADNPKNEDEVAQNMDMPQNAAGT
              |||| :| | :||| ||||| ||||| ||||| ||||| ||||| |||||
a287          VSGAPQADTQ--DATAGKGGQDMAAVSAENTGNGGAATTNDPENKDEGPONDMPQNAADT
              70      80      90      100      110

              110     120     130     140     150     160     169
m287.pep      DSSTPNHTPDENMLAGNMENQATDAGESSQPANQPDMANAADMQGGDDPSAGGQNAGNTA
              ||||| ||| : :| ||| ||||| ||||| ||||| ||||| ||||| |||||
a287          DSSTPNHTPAPNMPTRDMGNQAPDAGESAQPANQPDMANAADMQGGDDPSAG-ENAGNTA
              120     130     140     150     160     170

              170     180     190     200     210     220     229
m287.pep      AQGANQAGNNQAAGSSDPIPASNPAPANGGSNFRVLDLANGVLIDGPSQNTLTTHCKGDS
              |:||| |||:|:|:| :||| :|||:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|
a287          DQANQAENNQVGGSONPASSTNPATNGGSDFGRINVANGIKLDSGSENVTLTHCKDKV
              180     190     200     210     220     230

              230     240     250     260     270     280     289
m287.pep      CSGNNFLDEEVQLKSEFEKLSADAKISNYKKDKGKNDKFVGLVADSVQMKGINQYIIFYKP
              |: :|||:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|
a287          CD-RDFLDEEAPPKSEFEKLSDEEKINKYKKDEQRENFVGLVADRVEKNGTNKYVIIYKD
              240     250     260     270     280     290

              290     300     310     320     330     340
m287.pep      KP--TSFARFRRSARSRRLPAEMPLIPVQADTLIVDGEAVSLTGHSGNIFAPEGNRYR
              | :| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||

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a287      KSASSSSARFRRSARSRRSLPAEMPLIPVNQADTLIVDGEAVSLTGHSGNIFAPEGNYRY
           300      310      320      330      340      350

m287.pep  350      360      370      380      390      400
           LTYGAEKLPGGSYALRVQGEPAKGEMLAGAAVYNGEVLHFHTENGRPYPTRGRFAAKVDF
           ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
a287      LTYGAEKLSGGSYALSVMQGEPAKGEMLAGTAVYNGEVLHFHMENGRPSPSGGRFAAKVDF
           360      370      380      390      400      410

m287.pep  410      420      430      440      450      460
           GSKSVDGIIDSGDDLHMGTKQFKAAIDGNGFKGTWTENGSGDVSGKFYGPAGEEVAGKYS
           ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
a287      GSKSVDGIIDSGDDLHMGTKQFKAAIDGNGFKGTWTENGSGDVSGRFYGPAGEEVAGKYS
           420      430      440      450      460      470

m287.pep  470      480      489
           YRPTDAEKGGFGVFAGKKEQDX
           ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
a287      YRPTDAEKGGFGVFAGKKEQDX
           480      490

```

406

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 74>:

```

m406.seq
1   ATGCAAGCAC GGCTGCTGAT ACCTATTCTT TTTTCAGTTT TTATTTTATC
51  CGCCTGCGGG ACAC TGACAG GTATTCCATC GCATGGCGGA GGTAAACGCT
101 TTGCGGTCGA ACAAGAACTT GTGGCCGCTT CTGCCAGAGC TGCCGTTAAA
151 GACATGGATT TACAGGCATT ACACGGACGA AAAGTTGCAT TGTACATTGC
201 CACTATGGGC GACCAAGGTT CAGGCAGTTT GACAGGGGGT CGCTACTCCA
251 TTGATGCACT GATTCTGTGGC GAATACATAA ACAGCCCTGC CGTCCGTACC
301 GATTACACCT ATCCACGTTA CGAAACCACC GCTGAAACAA CATCAGGCGG
351 TTTGACAGGT TTAACCACTT CTTTATCTAC ACTTAATGCC CCTGCACTCT
401 CTGCGACCCA ATCAGACGGT AGCGGAAGTA AAAGCAGTCT GGGCTTAAAT
451 ATTGGCGGGA TGGGGGATTA TCGAAATGAA ACCTTGACGA CTAACCGCGG
501 CGACACTGCC TTTCTTTCCC ACTTGGTACA GACCGTATTT TTCTGCGGG
551 GCATAGACGT TGTCTTCTCCT GCCAATGCCG ATACAGATGT GTTTATTAAC
601 ATCGACGTAT TCGGAACGAT ACGCAACAGA ACCGAAATGC ACCTATACAA
651 TGCCGAAACA CTGAAAGCCC AAACAAAAC TGAATATTTC GCAGTAGACA
701 GAACCAATAA AAAATTGCTC ATCAAACCAA AAACCAATGC GTTTGAAGCT
751 GCCTATAAAG AAAATTACGC ATGTGTGATG GGGCCGTATA AAGTAAGCAA
801 AGGAATTAAA CCGACGGAAG GATTAATGGT CGATTTCTCC GATATCCGAC
851 CATACGGCAA TCATACGGGT AACTCGGCC CATCCGTAGA GGCTGATAAC
901 AGTCATGAGG GGTATGGATA CAGCGATGAA GTAGTGCAGC AACATAGACA
951 AGGACAACCT TGA

```

This corresponds to the amino acid sequence <SEQ ID 75; ORF 406>:

```

m406.pap
1   MQARLLIPIL FSVFILSACG TLTGIPSHGG GKRFAVEQEL VAASARAAVK
51  DMDLQALHGR KVALYIATMG DQSGSLTGG RYSIDALIRG EYINSPAVRT
101 DYTYPRIYET AETTSGLTGT LTSLSTLNA PALSRQSDG SGSSSLGLN
151 IGGMGDYRNE TLTTNPRDTA FLSHLVQTVF FLRGIDVVSF ANADTDVFIN
201 IDVFGTIRNR TEMHLYNAET LKAQTKLEYF AVDRTNKLL IKPKTNAFEA
251 AYKENYALWM GPYKVSQGIK PTEGLMVDFS DIRPYGNHTG NSAPSVEADN
301 SHEGYGYSDE VVRQHRQQQP *

```

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The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 76>:

```

g406.seq
  1  ATGCGGGCAC GGCTGCTGAT ACCTATTCTT TTTTCAGTTT TTATTTTATC
 51  CGCCTGCGGG ACACTGACAG GTATTCCATC GCATGGCGGA GGCAAACGCT
101  TCGCGGTCGA ACAAGAACTT GTGGCCGCTT CTGCCAGAGC TGCCGTTAA
151  GACATGGATT TACAGGCATT ACACGGACGA AAAGTTGCAT TGTACATTGC
201  AACTATGGGC GACCAAGGTT CAGGCAGTTT GACAGGGGGT CGCTACTCCA
251  TTGATGCACT GATTGCGGCG GAATACATAA ACAGCCCTGC CGTCGCGACC
301  GATTACACCT ATCCGCGTTA CGAAACCACC GCTGAAACAA CATCAGGCGG
351  TTTGACGGGT TTAACCACTT CTTTATCTAC ACTTAATGCC CCTGCACTCT
401  CGCGCACCCA ATCAGACGGT AGCGGAAGTA GGAGCAGTCT GGGCTTAAAT
451  ATTGGCGGGA TGGGGGATTA TCGAAATGAA ACCTTGACGA CCAACCCGCG
501  CGACACTGCC TTTCTTTCCC ACTTGGTGCA GACCGTATTT TTCCTGCGCG
551  GCATAGACGT TGTCTCTCCT GCCAATGCCG ATACAGATGT GTTTATTAA
601  ATCGACGTAT TCGGAACGAT ACGCAACAGA ACCGAAATGC ACCTATACAA
651  TGCCGAAACA CTGAAAGCCC AAACAAAACCT GGAATATTTT GCAGTAGACA
701  GAACCAATAA AAAATTGCTC ATCAAACCCA AAACCAATGC GTTGAAGCT
751  GCCTATAAAG AAAATTACGC ATTGTGGATG GGGCCGTATA AAGTAAGCAA
801  AGGAATCAAA CCGACGGAAG GATTGATGGT CGATTTCTCC GATATCCAAC
851  CATACGCGAA TCATACGGGT AACTCCGCC CATCCGTAGA GGCTGATAAC
901  AGTCATGAGG GGTATGGATA CAGCGATGAA GCAGTGCGAC AACATAGACA
951  AGGGCAACCT TGA

```

This corresponds to the amino acid sequence <SEQ ID 77; ORF 406.ng>:

```

g406.pep
  1  MRARLLIPIL FSVFILSACG TLTGIPSHGG GKRFAVEQEL VAASARAANK
 51  DMDLQALHGR KVALYIATMG DQSGSLTGG RYSIDALIRG EYINSPAVRT
101  DYTYPRIYET AETTSGLTGT LTSLSTLNA PALSRQSDG SGSRSSLGLN
151  IGGMGDYRNE TLTTNPRDTA FLSHLVQTVF FLRGIDVVSF ANADTDVFIN
201  IDVFGTIRNR TEMHLYNAET LKAQTKLEYF AVDRTNKKLL IKPKTNAFEA
251  AYKENYALWM GPYKVSKEGIK PTEGLMVDFF DIQPYGNHTG NSAPSVEADN
301  SHEGYGYSDE AVRQHRQGGP *

```

ORF 406.ng shows 98.8% identity over a 320 aa overlap with a predicted ORF (ORF406.a) from *N. gonorrhoeae*:

g406/m406

	10	20	30	40	50	60
g406.pep	MRARLLIPILFSVFILSACGTLTGIPSHGGGKRFAVEQELVAASARAANKDMDLQALHGR					
	:					
m406	MQARLLIPILFSVFILSACGTLTGIPSHGGGKRFAVEQELVAASARAANKDMDLQALHGR					
	10	20	30	40	50	60
	70	80	90	100	110	120
g406.pep	KVALYIATMGDQSGSLTGGGRYSIDALIRGEYINSPAVRTDYTPRIYETTAETTSGLTGT					
m406	KVALYIATMGDQSGSLTGGGRYSIDALIRGEYINSPAVRTDYTPRIYETTAETTSGLTGT					
	70	80	90	100	110	120
	130	140	150	160	170	180
g406.pep	LTSLSTLNAPALSRQSDGSGSRSSLGLNIGMGDYRNETLTNPRDTAFLSHLVQTVF					
	:					
m406	LTSLSTLNAPALSRQSDGSGSKSSLGLNIGMGDYRNETLTNPRDTAFLSHLVQTVF					
	130	140	150	160	170	180
	190	200	210	220	230	240

- 110 -

```

g406.pep  FLRGIDVVSPANADTDVFINIDVFGTIRNRTEMHLYNAETLKAQTKLEYFAVDRTNKKLL
|||||
m406      FLRGIDVVSPANADTDVFINIDVFGTIRNRTEMHLYNAETLKAQTKLEYFAVDRTNKKLL
          190      200      210      220      230      240

          250      260      270      280      290      300
g406.pep  IKPKTNAFEAAYKENYALWMGPYKVSCKIKPTEGLMVDFSIDIQPYGNHTGNSAPSVEADN
|||||
m406      IKPKTNAFEAAYKENYALWMGPYKVSCKIKPTEGLMVDFSIDIRPYGNHTGNSAPSVEADN
          250      260      270      280      290      300

          310      320
g406.pep  SHEGYGYSDEAVRQHRQGPX
|||||
m406      SHEGYGYSDEVVRQHRQGPX
          310      320

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 78>:

```

a406.seq
1   ATGCAAGCAC GGCTGCTGAT ACCTATTCTT TTTTCAGTTT TTATTTTATC
51  CGCCTGCGGG AACTGACAG GTATTCCATC GCATGGCGGA GGTAAACGCT
101 TCGCGGTCGA ACAAGAACTT GTGGCCGCTT CTGCCAGAGC TGCCGTTAAA
151 GACATGGATT TACAGGCATT ACACGGACGA AAAGTTGCAT TGTACATTGC
201 AACTATGGGC GACCAAGGTT CAGGCAGTTT GACAGGGGT CGCTACTCCA
251 TTGATGCACT GATTCGTGGC GAATACATAA ACAGCCCTGC CGTCCGTACC
301 GATTACACCT ATCCACGTTA CGAAACCACC GCTGAAACAA CATCAGGCGG
351 TTTGACAGGT TTAACCACTT CTTTATCTAC ACTTAATGCC CCTGCACTCT
401 CGCGCACCCA ATCAGACGGT AGCGGAAGTA AAAGCAGTCT GGGCTTAAAT
451 ATTGGCGGGA TGGGGGATTA TCGAAATGAA ACCTTGACGA CTAACCCGCG
501 CGACACTGCC TTTCTTTCCC ACTTGGTACA GACCGTATTT TTCCTGCGCG
551 GCATAGACGT TGTTTCTCCT GCCAATGCCG ATACGGATGT GTTTATTAAC
601 ATCGAGTAT TCGGAACGAT ACGCAACAGA ACCGAAATGC ACCTATACAA
651 TGCCGAAACA CTGAAAGCCC AAACAAAACCT GGAATATTTC GCAGTAGACA
701 GAACCAATAA AAAATTGCTC ATCAAACCAA AAACCAATGC GTTTGAAGCT
751 GCCTATAAAG AAAATTACGC ATTGTGGATG GGACCGTATA AAGTAAGCAA
801 AGGAATTAA CCGACAGAAG GATTAATGGT CGATTCTCC GATATCCAAC
851 CATACGCAA TCATATGGGT AACTCTGCCC CATCCGTAGA GGCTGATAAC
901 AGTCATGAGG GGTATGGATA CAGCGATGAA GCAGTGCAC GACATAGACA
951 AGGCAACCT TGA

```

This corresponds to the amino acid sequence <SEQ ID 79; ORF 406.a>:

```

a406.pep
1   MQARLLIPIL FSVFILSACG TLTGIPSHGG GKRFAVEQEL VAASARAAVK
51  DMDLQALHGR KVALYIATMG DQSGSLTGG RYSIDALIRG EYINSPAVRT
101 DYTYPYRNETT AETTSGLTG LTSLSTLNA PALSRTQSDG SGSSSLGLN
151 IGGMGDYRNE TLTNPRDTA FLSHLVQTVF FLRGIDVVSP ANADTDVFIN
201 IDVFGTIRNR TEMHLYNAET LKAQTKLEYF AVDRTNKKLL IKPKTNAFEA
251 AYKENYALWM GPYKVSCKIK PTEGLMVDFS DIQPYGNHMG NSAPSVEADN
301 SHEGYGYSDE AVRRHRQGP *

m406/a406  ORFs 406 and 406.a showed a 98.8% identity in 320 aa overlap

          10      20      30      40      50      60
m406.pep  MQARLLIPILFSVFILSACGTLTGIPSHGGGKRFAVEQELVAASARAAVKDMDLQALHGR
|||||
a406      MQARLLIPILFSVFILSACGTLTGIPSHGGGKRFAVEQELVAASARAAVKDMDLQALHGR
          10      20      30      40      50      60

          70      80      90      100     110     120
m406.pep  KVALYIATMGDQSGSLTGGRYSIDALIRGEYINSPAVRTDYTYPRYETTAETTSGLTG
|||||

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a406	KVALYIATMGDQGSGLTGGRYSIDALIRGEYINSPAVRTDYTPRYETTAETTSGLTG
	70 80 90 100 110 120
m406.pep	130 140 150 160 170 180
	LTSSLSTLNAPALSRTQSDGSGSKSSLGLNIGGMGDYRNETLTNPRDTAFLSHLVQTVF
a406	LTSSLSTLNAPALSRTQSDGSGSKSSLGLNIGGMGDYRNETLTNPRDTAFLSHLVQTVF
	130 140 150 160 170 180
m406.pep	190 200 210 220 230 240
	FLRGIDVVSPANADTDVFINIDVFGTIRNRTEMHLYNAETLKAQTKLEYFAVDRTNKKLL
a406	FLRGIDVVSPANADTDVFINIDVFGTIRNRTEMHLYNAETLKAQTKLEYFAVDRTNKKLL
	190 200 210 220 230 240
m406.pep	250 260 270 280 290 300
	IKPKTNAFEAAYKENYALWMGPYKVS KG I KPTEGLMVDFSDIRPYGNHTGNSAPSVEADN
a406	IKPKTNAFEAAYKENYALWMGPYKVS KG I KPTEGLMVDFSDIQPYGNHMGNSAPSVEADN
	250 260 270 280 290 300
m406.pep	310 320
	SHEGYGYSDEVVRQHRQGPX
a406	SHEGYGYSDEAVRRHRQGPX
	310 320

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 80>:

m726.seq

1	ATGACCATCT	ATTTCAAAAA	CGGCTTTTAC	GACGACACAT	TGGGCGGCAT
51	CCCCGAAGGC	GCGGTTGCCG	TCCGCGCCGA	AGAATACGCC	GCCCTTTTGG
101	CAGGACAGGC	GCAGGGCGGG	CAGATTGCCG	CAGATTCCGA	CGGCCGCCCC
151	GTTTTAACCC	CGCCGCGCCC	GTCCGATTAC	CACGAATGGG	ACGGCAAAAA
201	ATGGAAAATC	AGCAAAGCCG	CCGCCGCCGC	CCGTTTCGCC	AAACAAAAAA
251	CCGCCTTGGC	ATTCCGCCTC	GCGGAAAAGG	CGGACGAACT	CAAAAACAGC
301	CTCTTGCGCG	GCTATCCCCA	AGTGGAAATC	GACAGCTTTT	ACAGGCAGGA
351	AAAAGAAGCC	CTCGCGCGGC	AGGCGGACAA	CAACGCCCCG	ACCCCGATGC
401	TGGCGCAAAT	CGCCGCCGCA	AGGGGCGTGG	AATTGGACGT	TTTGATTGAA
451	AAAGTTATCG	AAAAATCCGC	CCGCCTGGCT	GTTGCCGCCG	GCGCGATTAT
501	CGGAAAGCGT	CAGCAGCTCG	AAGACAAATT	GAACACCATC	GAAACCGCGC
551	CCGATTGGA	CGCGCTGGAA	AAGGAAATCG	AAGAATGGAC	GCTAAACATC
601	GGCTGA				

This corresponds to the amino acid sequence <SEQ ID 81; ORF 726>:

m726.pep

1	MTIYFKNGFY	DDTLGGIPEG	AVAVRAEEYA	ALLAQOAGG	QIAADSDGRP
51	VLTPRPSPDY	HEWDGKKWKI	SKAAAAARFA	KQKTALAFRL	AEKADELKNS
101	LLAGYPQVEI	DSFYRQEKEA	LARQADNNAP	TPMLAQIAAA	RGVELDVLI
151	KVIEKSARLA	VAAGAIIGKR	QLEDKLNTI	ETAPGLDALE	KEIEEWTINI
201	G*				

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 82>:

m907-2.seq

1	ATGAGAAAAC	CGACCGATAC	CCTACCCGTT	AATCTGCAAC	GCCGCCGCCCT
51	GTTGTGTGCC	GCCGGTGCGT	TGTTGCTCAG	TCCTCTGGCG	CACGCCGGCG
101	CGCAACGTGA	GGAAACGCTT	GCCGACGATG	TGGCTTCCGT	GATGAGGAGT

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151 TCTGTCGGCA GCGTCAATCC GCCGAGGCTG GTGTTTGACA ATCCGAAAGA
201 GGGCGAGCGT TGGTTGTCTG CCATGTCGGC ACGTTTGGA AGGTTTCGTCC
251 CCGAGGAGGA GGAGCGGCGC AGGCTGCTGG TCAATATCCA GTACGAAAGC
301 AGCCGGGCGG GTTTGGATAC GCAGATTGTG TTGGGGCTGA TTGAGGTGGA
351 AAGCGCGTTC CGCCAGTATG CAATCAGCGG TGTCGGCGCG CGCGGCCTGA
401 TGCAGGTTAT GCCGTTTTGG AAAAACTACA TCGGCAAACC GGCGCACAAAC
451 CTGTTTCGACA TCCGCACCAA CCTGCGTTAC GGCTGTACCA TCCTGCGCCA
501 TTACCGGAAT CTTGAAAAAG GCAACATCGT CCGCGCGCTT GCCCGCTTTA
551 ACGGCAGCTT GGGCAGCAAT AAATATCCGA ACGCCGTTTT GGGCGCGTGG
601 CGCAACCGCT GGCAGTGCGG TTGA

```

This corresponds to the amino acid sequence <SEQ ID 83; ORF 907-2>:

m907-2.pep

```

1 MRKPTDTLPV NLQRRRLCA AGALLLSPLA HAGAQREETL ADDVASVMRS
51 SVGSVNPPRL VFDNPKGER WLSAMSARLA RFVPEEEERR RLLVNIQYES
101 SRAGLDTQIV LGLIEVESAF RQYAISGVA RGLMQVMPFW KNYIGKPAHN
151 LFDIRTNLRY GCTILRHRYN LEKGNIVRAL ARFNGSLGSN KYPNAVLGAW
201 RNRWQWR*

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 84>:

m953.seq

```

1 ATGAAAAAAA TCATCTTCGC CGCACTCGCA GCCGCCGCCA TCAGTACTGC
51 CTCCGCCGCC ACCTACAAAG TGGACGAATA TCACGCCAAC GCCCGTTTCG
101 CCATCGACCA TTTCAACACC AGCACCAACG TCGGCGGTTT TTACGGTCTG
151 ACCGGTTCCG TCGAGTTCGA CCAAGCAAAA CGCGACGGTA AAATCGACAT
201 CACCATCCCC ATTGCCAACC TGCAAAGCGG TTCGCAACAC TTTACCGACC
251 ACCTGAAATC AGCCGACATC TTCGATGCCG CCAATATATC GGACATCCGC
301 TTTGTTTCCA CCAAATTCOA CTTCAACGGC AAAAACTGG TTTCCGTTGA
351 CGGCAACCTG ACCATGCACG GCAAAACCGC CCCCCTCAA CTCAAAGCCG
401 AAAAAATCAA CTGCTACCAA AGCCCGATGG AGAAAACCGA AGTTTGTGGC
451 GCGGACTTCA GCACCACCAT CGACCGCACC AAATGGGGCA TGGACTACCT
501 CGTTAACGTT GGTATGACCA AAAGCGTCCG CATCGACATC CAAATCGAGG
551 CAGCCAAACA ATAA

```

This corresponds to the amino acid sequence <SEQ ID 85; ORF 953>:

m953.pep

```

1 MKKIIFAALA AAAISTASAA TYKVDEYHAN ARFAIDHFNT STNVGGFYGL
51 TGSVEFDQAK RDGKIDITIP IANLQSGSQH FTDHLKSADI FDAAQYPDIL
101 FVSTKFNENG KKLVSVDGNL TMHGKTAPVK LKAEKFNCYQ SPMEKTEVCG
151 GDFSTTIDRT KWGMDYLVNV GMTKSVRIDI QIEAAKQ*

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 86>:

orf1-1.seq

```

1 ATGAAAACAA CCGACAAACG GACAACCGAA ACACACCGCA AAGCCCCGAA
51 AACC GGCCGC ATCCGCTTCT CGCCTGCTTA CTTAGCCATA TGCCTGTCGT
101 TCGGCATTCT TCCCCAAGCC TGGGCGGGAC ACCTTATTTT CGGCATCAAC
151 TACCAATACT ATCGCGACTT TGCCGAAAAT AAAGGCAAGT TTGCAGTCGG
201 GGCAGAAAGAT ATTGAGTTT ACAACAAAAA AGGGGAGTTG GTCGGCAAAT
251 CAATGACAAA AGCCCCGATG ATTGATTTTT CTGTGGTGTC GCGTAACGGC
301 GTGGCGGCAT TGGTGGGCGA TCAATATATT GTGAGCGTGG CACATAACGG
351 CGGCTATAAC AACGTTGATT TTGGTGCGGA AGGAAGAAAT CCCGATCAAC
401 ATCGTTTTAC TTATAAAATT GTGAAACGGA ATAATTATAA AGCAGGGACT
451 AAAGGCCATC CTTATGGCGG CGATTATCAT ATGCCGCGTT TGCATAAATT
501 TGTACAGAT GCAGAACCTG TTGAAATGAC CAGTTATATG GATGGGCGGA

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551 AATATATCGA TCAAAATAAT TACCCTGACC GTGTTTCGTAT TGGGGCAGGC
601 AGGCAATATT GGCGATCTGA TGAAGATGAG CCCAATAACC GCGAAAGTTC
651 ATATCATATT GCAAGTGCGT ATTCTTGGCT CGTTGGTGGC AATACCTTTG
701 CACAAATGG ATCAGGTGGT GGCACAGTCA ACTTAGGTAG TGA AAAAAT
751 AAACATAGCC CATATGGTTT TTTACCAACA GGAGGCTCAT TTGGCGACAG
801 TGGCTCACCA ATGTTTATCT ATGATGCCCA AAAGCAAAAG TGGTTAATTA
851 ATGGGGTATT GCAAACGGGC AACCCCTATA TAGGAAAAAG CAATGGCTTC
901 CAGCTGGTTC GTAAAGATTG GTTCTATGAT GAAATCTTTG CTGGAGATAC
951 CCATTTCAGTA TTCTACGAAC CACGTCAAAA TGGGAAATAC TCTTTTAACG
1001 ACGATAATAA TGGCACAGGA AAAATCAATG CCAAACATGA ACACAATTCT
1051 CTGCCTAATA GATTA AAAAAC ACGAACCGTT CAATTGTTTA ATGTTTCTTT
1101 ATCCGAGACA GCAAGAGAAC CTGTTTATCA TGCTGCAGGT GGTGTCAACA
1151 GTTATCGACC CAGACTGAAT AATGGAGAAA ATATTTCTTT TATTGACGAA
1201 GGAAGAGGCG AATTGATACT TACCAGCAAC ATCAATCAAG GTGCTGGAGG
1251 ATTATATTTT CAAGGAGATT TTACGGTCTC GCCTGAAAAT AACGAACTT
1301 GGCAAGGCGC GGGCGTTCAT ATCAGTGAAG ACAGTACCGT TACTTGGAAA
1351 GTAAACGGCG TGGCAAACGA CCGCTGTCC AAAATCGGCA AAGGCACGCT
1401 GCACGTTCAA GCCAAAGGGG AAAACCAAGG CTCGATCAGC GTGGGCGACG
1451 GTACAGTCAT TTTGGATCAG CAGGCAGACG ATAAAGGCAA AAAACAAGCC
1501 TTTAGTGAAA TCGGCTTGGT CAGCGGCAGG GGTACGGTGC AACTGAATGC
1551 CGATAATCAG TTCAACCCCG ACAAACCTCTA TTTCCGGCTTT CGCGGCGGAC
1601 GTTTGGATTT AAACGGGCAT TCGCTTTCGT TCCACCGTAT TCAAAATACC
1651 GATGAAGGGG CGATGATTGT CAACCACAAT CAAGACAAAG AATCCACCGT
1701 TACCATTACA GGCAATAAAG ATATTGCTAC AACCGCAAT AACAAACAGT
1751 TGGATAGCAA AAAAGAAATT GCCTACAACG GTTGGTTTGG CGAGAAAGAT
1801 ACGACCAAAA CGAACGGGCG GCTCAACCTT GTTTACCAGC CCGCCGAGA
1851 AGACCGCACC CTGCTGCTTT CCGCGGGAAC AAATTTAAAC GGCAACATCA
1901 CGCAACAAA CGGCAACTG TTTTCAGCG GCAGACCAAC ACCGCACGCC
1951 TACAATCATT TAAACGACCA TTGGTCGCAA AAAGAGGGCA TTCCTCGCGG
2001 GGAAATCGTG TGGGACAACG ACTGGATCAA CCGCACATTT AAAGCGGAAA
2051 ACTTCCAAAT TAAAGCGGGA CAGGCGGTGG TTTCCGCAA TGTTGCCAAA
2101 GTGAAAGGCG ATTGGCATT T GAGCAATCAC GCCCAAGCAG TTTTGGTGT
2151 CGCACCGCAT CAAAGCCACA CAATCTGTAC ACGTTCGGAC TGGACGGGTC
2201 TGACAAATTG TGTCGAAAAA ACCATTACCG ACGATAAAGT GATTGCTTCA
2251 TTGACTAAGA CCGACATCAG CCGCAATGTC GATCTTGCCG ATCAGCTCA
2301 TTTAAATCTC ACAGGGCTTG CCACACTCAA CCGCAATCTT AGTGCAAATG
2351 GCGATACACG TTATACAGT AGCCACAACG CCACCAAAA CGGCAACCTT
2401 AGCCTCGTGG GCAATGCCCA AGCAACATTT AATCAAGCCA CATTAAACGG
2451 CAACACATCG GCTTCGGGCA ATGCTTCATT TAATCTAAGC GACCACGCCG
2501 TACAAAACGG CAGTCTGACG CTTCCGGCA ACGCTAAGGC AAACGTAAGC
2551 CATTCCGCAC TCAACGGTAA TGCTCCCTA GCCGATAAGG CAGTATTCCA
2601 TTTTGAAAGC AGCCGCTTTA CCGGACAAAT CAGCGGCGGC AAGGATACGG
2651 CATTACACTT AAAAGACAGC GAATGGACGC TGCCGTCAGG CACGGAATTA
2701 GGCAATTTAA ACCTTGACAA CGCCACCATT ACACTCAATT CCGCTATCG
2751 CCACGATGCG GCAGGGGCGC AAACCGGCAG TGCGACAGAT GCGCCGCGCC
2801 GCGGTTGCGC CCGTTCGCGC CGTTCCTAT TATCCGTTAC ACCGCCAAT
2851 TCGGTAGAAT CCCGTTTCAA CACGCTGACG GTAAACGGCA AATTGAACGG
2901 TCAGGGAACA TTCCGCTTTA TGTCGGAAC CTTCGGCTAC CGCAGCGACA
2951 AATTGAAGCT GCGGGAAGT TCCGAAGGCA CTACACCTT GCGGTCAAC
3001 AATACCGGCA ACGAACCTGC AAGCCTCGAA CAATTGACGG TAGTGGAAGG
3051 AAAAGACAAC AAACCGCTGT CCGAAAACCT TAATTTACC CTGCAAAACG
3101 AACACGTCGA TGCCGGCGCG TGGCGTTACC AACTCATCCG CAAAGACGGC
3151 GAGTTCCGCC TGCATAATCC GGTCAAAGAA CAAGAGCTTT CCGACAACT
3201 CCGCAAGGCA GAAGCCAAAA AACAGGCGGA AAAAGACAAC GCGCAAGGCC
3251 TTGACGCGT GATTGCGGCC GGGCGCGATG CCGTCGAAAA GACAGAAAGC
3301 GTTGCCGAAC CGGCCCAGCA GGCAGGCGGG GAAAATGTCG GCATTATGCA
3351 GGCGGAGGAA GAGAAAAAAC GGGTGCAGGC GGATAAAGAC ACCGCCTTGG
3401 CGAAACAGCG CGAAGCGGAA ACCCGGCCGG CTACCACCGC CTCCCCCGC
3451 CCCCGCCGCG CCCGCGGGA TTTGCCGCAA CTGCAACCCC AACCGCAGCC
3501 GCAACCGCAG CGCGACCTGA TCAGCCGTTA TGCCAATAGC GGTTCGAGT
3551 AATTTTCCGC CACGCTCAAC AGCGTTTTCG CCGTACAGGA CGAATTAGAC
3601 CGCGTATTTG CCGAAGACCG CCGCAACGCC GTTTGGACAA GCGGCATCCG
3651 GGACACCAAA CACTACCGTT CGCAAGATTT CCGCGCTAC CGCCAACAAA

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3701 CCGACCTGCG CCAAATCGGT ATGCAGAAAA ACCTCGGCAG CGGGCGCGTC
3751 GGCATCCTGT TTTCGCACAA CCGGACCGAA AACACCTTCG ACGACGGCAT
3801 CGGCAACTCG GCACGGCTTG CCCACGGCGC CGTTTTCGGG CAATACGGCA
3851 TCGACAGGTT CTACATCGGC ATCAGCGCGG GCGCGGGTTT TAGCAGCGGC
3901 AGCCTTTCAG ACGGCATCGG AGGCAAAATC CGCCGCCGCG TGCTGCATTA
3951 CGGCATTTCAG GCACGATACC GCGCCGGTTT CGGCGGATTG GGCATCGAAC
4001 CGCACATCGG CGCAACGCGC TATTTTCGTCC AAAAAGCGGA TTACCGCTAC
4051 GAAAACGTCA ATATCGCCAC CCCCGGCCTT GCATTCAACC GCTACCGCGC
4101 GGGCATTAAAG GCAGATTATT CATTCAAACC GCGCAACAC ATTTCATCA
4151 CGCCTTATTT GAGCCTGTCC TATACCGATG CCGCTTCGGG CAAAGTCCGA
4201 ACACGCGTCA ATACCGCCGT ATTGGCTCAG GATTTCGGCA AAACCGCAG
4251 TGCGGAATGG GGCCTAAACG CCGAAATCAA AGGTTTCACG CTGTCCCTCC
4301 ACGCTGCCGC CGCAAAGGC CCGCAACTGG AAGCGCAACA CAGCGCGGGC
4351 ATCAATTAG GCTACCGCTG GTAA

```

This corresponds to the amino acid sequence <SEQ ID 87; ORF orf1-1>:

## orf1-1.pep

```

1 MKTTDKRTE THRKAPKTGR IRFSPAYLAI CLSFGILPQA WAGHTYFGIN
51 YQYYRDFAEK KGKFAVGAKD IEVYNKKGEL VGKSMTKAPM IDFSVSVSRNG
101 VAALVGQYI VSVAHNGGYN NVDFGAEGRN PDQHRFTYKI VKRNNYKAGT
151 KGHYPYGGDYH MPRLHKFVTD AEPVEMTSYM DGRKYIDQNN YPDRVRIGAG
201 RQYWRSDEDE PNNRESSYHI ASAYSWLVGG NTFAQNGSGG GTVNLGSEKI
251 KHSPYGFLEPT GGSFGDSGSP MFIYDAQKQK WLINGVLQTG NPYIGKSNFG
301 QLVKRWDFYD EIFAGDTHSV FYEPRQNGKY SFNDNNGTG KINAKHEHNS
351 LPNRLKTRTV QLFNVSLSET AREPVYHAAG GVNRYRPRLN NGENISFIDE
401 GKGEILITSN INQAGGLYF QGDFTVSPEN NETWQAGVH ISEDSTVTWK
451 VNGVANDRLS KIGKGLHVQ AKGENQGSIS VGDGTVILDQ QADDKGGKQA
501 FSEIGLVSGR GTVQLNADNQ FNPDKLYFGF RGGRLDLNGH SLSFHRIQNT
551 DEGAMIVNHN QDKESTVTIT GNKDIATTGN NNSLDSKKEI AYNGWFGKED
601 TTKTNGRLNL VYQPAEDRT LLLSGGTNLN GNITQTNGKL FFSGRPTPHA
651 YNHLNDHWSQ KEGIPRGEIV WDNDWINRTF KAENFQIKGG QAVVSRNVAK
701 VKGDWHLNSH AQAVFVAPH QSHTICTRSD WTGLTNCVEK TITDDKVIAS
751 LTKTDISGNV DLADHAHLNL TGLATLNGNL SANGDTRYTV SHNATQNGNL
801 SLVGNAQATF NQATLNGNTS ASGNASFNLS DHAVQNGSLT LSGNAKANVS
851 HSALNGNVSL ADKAVFHFES SRFTGQISGG KDTALHLKDS EWTLPSTEL
901 GNLNLDNATI TLNSAYRHDA AGAQTGSATD APRRRSRRSR RSLLSVTPPT
951 SVESRENTLT VNGKLNGQGT FREMSELFY RSDKLKLAES SEGTYTLAVN
1001 NTGNEPASLE QLTVEGKDN KPLSENLFNT LQNEHVDAGA WRYQLIRKDG
1051 EFRHLNPKVE QELSDKLGKA EAKKQAEKDN AQSLDALIAA GRDAVEKTES
1101 VAEPARQAGG ENVGIMQAE EKKRVQADKD TALAKQREAE TRPATTAFFR
1151 ARRARRDLPO LQPQPQPQPQ RDLISRYANS GLSEFSATLN SVFAVQDELD
1201 RVFAEDRRNA VWTSGIRDIT HYRSQDFRAY RQOTDLRQIG MQKNLGSGRV
1251 GILFSHNRTE NTFDDGIGNS ARLAHGAVFG QYGIDRFYIG ISAGAGFSSG
1301 SLSDGIGGKI RRRVLHYGIQ ARYRAGFGGF GIEPHIGATR YFVQKADYRY
1351 ENVNIATPGL AFNRYRAGIK ADYSEKPAQH ISITPYLSLS YTDAAAGKVR
1401 TRVNTAVLAQ DFGKTRSAEW GVNAEIKGFT LSLHAAAAGK PQLEAQHSAG
1451 IKLGYRW*

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 88>:

## orf46-2.seq

```

1 TTGGGCATTT CCCGCAAAAT ATCCCTTATT CTGTCCATAC TGGCAGTGTG
51 CCTGCCGATG CATGCACACG CCTCAGATTT GGCAAAACGAT TCTTTTATCC
101 GGCAGGTTCT CGACCGTCAG CATTTCGAAC CCGACGGGAA ATACCACCTA
151 TTCGGCAGCA GGGGGGAACT TGCCGAGCGC AGCGGCCATA TCGGATTGGG
201 AAAAATACAA AGCCATCAGT TGGGCAACCT GATGATTCAA CAGGCGGCCA
251 TTAAAGGAAA TATCGGCTAC ATTGTCCGCT TTCCGATCA CGGGCACGAA
301 GTCCATTCCC CCTTCGACAA CCATGCCTCA CATTCCGATT CTGATGAAGC
351 CGGTAGTCCC GTTGACGGAT TTAGCCTTTA CCGCATCCAT TGGGACGGAT

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401  ACGAACACCA TCCCGCCGAC GGCTATGACG GGCCACAGGG CGGCGGCTAT
451  CCCGCTCCCA AAGGCGCGAG GGATATATAC AGCTACGACA TAAAAGGCGT
501  TGCCCAAAAT ATCCGCCTCA ACCTGACCGA CAACCGCAGC ACCGGACAAC
551  GGCTTGCCGA CCGTTTCCAC AATGCCGGTA GTATGCTGAC GCAAGGAGTA
601  GGGCAGCGAT TCAAACGCGC CACCCGATAC AGCCCCGAGC TGGACAGATC
651  GGGCAATGCC GCCGAAGCCT TCAACGGCAC TGCAGATATC GTTAAAAACA
701  TCATCGGCGC GGCAGGAGAA ATTGTGGCGC CAGGCGATGC CGTGAGGGGC
751  ATAAGCGAAG GCTCAAACAT TGCTGTCATG CACGGCTTGG GTCTGCTTTC
801  CACCGAAAAC AAGATGGCGC GCATCAACGA TTTGGCAGAT ATGGCGCAAC
851  TCAAAGACTA TGCCGCAGCA GCCATCCGCG ATTGGGCAGT CCAAAACCCC
901  AATGCCGCAC AAGGCATAGA AGCCGTCAGC AATATCTTTA TGGCAGCCAT
951  CCCCATCAAA GGGATTGGAG CTGTTCCGGG AAAATACGGC TTGGGCGGCA
1001 TCACGGCACA TCCTATCAAG CGGTTCGAGA TGGGCGCGAT CGCATTGCCG
1051 AAAGGGAAAT CCGCCGTCAG CGACAATTTT GCCGATGCGG CATACGCCAA
1101 ATACCCGTCC CCTTACCATT CCCGAAATAT CCGTTCAAAC TTGGAGCAGC
1151 GTTACGGCAA AGAAAACATC ACCTCCTCAA CCGTGCCGCC GTCAAACGGC
1201 AAAAATGTCA AACTGGCAGA CCAACGCCAC CCGAAGACAG GCGTACCGTT
1251 TGACGGTAAA GGGTTTCCGA ATTTTGAGAA GCACGTGAAA TATGATACGA
1301 AGCTCGATAT TCAAGAATTA TCGGGGGGCG GTATACCTAA GGCTAAGCCT
1351 GTGTTTGATG CGAAACCGAG ATGGGAGGTT GATAGGAAGC TTAATAAATT
1401 GACAACTCGT GAGCAGGTGG AGAAAAATGT TCAGGAAATA AGGAACGGTA
1451 ATATAAACAG TAACTTTAGC CAACATGCTC AACTAGAGAG GGAAATTAAT
1501 AAATAAAAT CTGCCGATGA AATTAATTTT GCAGATGGAA TGGGAAAATT
1551 TACCGATAGC ATGAATGACA AGGCTTTTAG TAGGCTTGTG AAATCAGTTA
1601 AAGAGAATGG CTTACAAAAT CCAGTTGTGG AGTACGTTGA AATAAATGGA
1651 AAAGCATATA TCGTAAGAGG AAATAATRGG GTTTTGTCTG CAGAATACCT
1701 TGGCAGGATA CATGAATTAA AATTTAAAAA AGTTGACTTT CCTGTTCTTA
1751 ATACTAGTTG GAAAAATCCT ACTGATGTCT TGAATGAATC AGGTAATGTT
1801 AAGAGACCTC GTTATAGGAG TAAATAA

```

This corresponds to the amino acid sequence <SEQ ID 89; ORF orf46-2>:

**orf46-2.pap**

```

1  LGISRKISLI LSILAVCLPM HAHASDLAND SFIRQVLDRO HFEPDGKYHL
51  FGSRGELAER SGHIGLGKIQ SHQLGNLMIQ QAAIKGNIGY IVRFSDHGHE
101 VHSPFDNHAS HSDSDEAGSP VDGFSLYRIH WDGYEHHPAD GYDGPQGGGY
151 PAPKGARDIY SYDIKGVQON IRLNLTNRS TGQRLADRFH NAGSMLTQGV
201 GDGFKRATRY SPELDRSNA AEFNGTADI VKNIIGAAGE IVGAGDAVQG
251 ISEGSNIAVM HGLGLLSTEN KMARINDLAD MAQLKDYAAA AIRDWAVQNP
301 NAAQGIEAVS NIFMAAIPK GIGAVRGKYG LGGITAHPIK RSQMGAIALP
351 KGKSAVSDNF ADAAYAKYPS PYHSRNIRSN LEQRYGKENI TSSTVPPSNG
401 KNVKLADQRH PKTGVPFDGK GFPNFEKHVK YDTKLDIQEL SGGGIPKAKP
451 VFDAKPRWEV DRKLNKLTR EQVEKNVQEI RNGNINSNFS QHAQLEREIN
501 KLKSADEINF ADGMGKFTDS MNDKAFSRLV KSVKENGFTN PVVEYVEING
551 KAYIVRGNNR VFAAEYLGRI HELKFKKVD FVPNTSWKNP TDVLNESGNV
601 KRPRYRSK*

```

Using the above-described procedures, the following oligonucleotide primers were employed in the polymerase chain reaction (PCR) assay in order to clone the ORFs as indicated:

**Olig nucleotides used for PCR**

Table 1

ORF	Primer	Sequence	Restriction sites
279	Forward	CGCGGATCCCATATG-TTGCTGCAATCACGATT <SEQ ID 90>	BamHI-NdeI
	Reverse	CCCGCTCGAG-TTTAGAAGCGGGCGGCAA <SEQ ID 91>	XhoI
519	Forward	CGCGGATCCCATATG-TTCAAATCCTTTGTCGTCA <SEQ ID 92>	BamHI-NdeI
	Reverse	CCCGCTCGAG-TTTGGCGGTTTGTCTGC <SEQ ID 93>	XhoI
576	Forward	CGCGGATCCCATATG-GCCGCCCCCGCATCT <SEQ ID 94>	BamHI-NdeI
	Reverse	CCCGCTCGAG-ATTTACTTTTTTGATGTGCGAC <SEQ ID 95>	XhoI
919	Forward	CGCGGATCCCATATG-TGCCAAAGCAAGAGCATC <SEQ ID 96>	BamHI-NdeI
	Reverse	CCCGCTCGAG-CGGGCGGTATTCGGG <SEQ ID 97>	XhoI
121	Forward	CGCGGATCCCATATG-GAAACACAGCTTTACAT <SEQ ID 98>	BamHI-NdeI
	Reverse	CCCGCTCGAG-ATAATAATATCCCGCGCCC <SEQ ID 99>	XhoI
128	Forward	CGCGGATCCCATATG-ACTGACAACGCACT <SEQ ID 100>	BamHI-NdeI
	Reverse	CCCGCTCGAG-GACCGCGTTGTGCGAAA <SEQ ID 101>	XhoI
206	Forward	CGCGGATCCCATATG-AAACACCGCCAACCGA <SEQ ID 102>	BamHI-NdeI
	Reverse	CCCGCTCGAG-TTCTGTAAAAAAGTATGTGC <SEQ ID 103>	XhoI
287	Forward	CCGGAATTCTAGCTAGC-CTTTCAGCCTGCGGG <SEQ ID 104>	EcoRI-NheI
	Reverse	CCCGCTCGAG-ATCCTGCTCTTTTTTGCC <SEQ ID 105>	XhoI
406	Forward	CGCGGATCCCATATG-TGCGGGACACTGACAG <SEQ ID 106>	BamHI-NdeI
	Reverse	CCCGCTCGAG-AGGTTGTCCTTGTCTATG <SEQ ID 107>	XhoI

## EXAMPLE 2

Expression of ORF 919

The primer described in Table 1 for ORF 919 was used to locate and clone ORF 919. The predicted gene 919 was cloned in pET vector and expressed in *E. coli*. The product of

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protein expression and purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 919-His fusion protein purification. Mice were immunized with the purified 919-His and sera were used for Western blot (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Symbols: M1, molecular weight marker; PP, purified protein, TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 919 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 919 are provided in Figure 10. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 919 and the amino acid sequence encoded thereby is provided in Example 1.

### EXAMPLE 3

#### Expression of ORF 279

The primer described in Table 1 for ORF 279 was used to locate and clone ORF 279. The predicted gene 279 was cloned in pGex vector and expressed in *E. coli*. The product of protein expression and purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 279-GST purification. Mice were immunized with the purified 279-GST and sera were used for Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 279 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 279 are provided in Figure 11. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 279 and the amino acid sequence encoded thereby is provided in Example 1.

## EXAMPLE 4

Expression of ORF 576

The primer described in Table 1 for ORF 576 was used to locate and clone ORF 576. The predicted gene 576 was cloned in pGex vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 576-GST fusion protein purification. Mice were immunized with the purified 576-GST and sera were used for Western blot (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B).. These experiments confirm that ORF 576 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 576 are provided in Figure 12. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 576 and the amino acid sequence encoded thereby is provided in Example 1.

## EXAMPLE 5

Expression of ORF 519

The primer described in Table 1 for ORF 519 was used to locate and clone ORF 519. The predicted gene 519 was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 519-His fusion protein purification. Mice were immunized with the purified 519-His and sera were used for Western blot (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 519 is a surface-exposed protein

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and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 519 are provided in Figure 13. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 519 and the amino acid sequence encoded thereby is provided in Example 1.

### EXAMPLE 6

#### Expression of ORF 121

The primer described in Table 1 for ORF 121 was used to locate and clone ORF 121. The predicted gene *121* was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 121-His fusion protein purification. Mice were immunized with the purified 121-His and sera were used for Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Results show that 121 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 121 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 121 are provided in Figure 14. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 121 and the amino acid sequence encoded thereby is provided in Example 1.

### EXAMPLE 7

#### Expression of ORF 128

The primer described in Table 1 for ORF 128 was used to locate and clone ORF 128. The predicted gene *128* was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 128-His purification. Mice were immunized with the purified 128-His and sera were used for

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Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D) and ELISA assay (panel E). Results show that 128 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 128 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 128 are provided in Figure 15. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 128 and the amino acid sequence encoded thereby is provided in Example 1.

#### EXAMPLE 8

##### Expression of ORF 206

The primer described in Table 1 for ORF 206 was used to locate and clone ORF 206. The predicted gene 206 was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 206-His purification. Mice were immunized with the purified 206-His and sera were used for Western blot analysis (panel B). It is worthnoting that the immunoreactive band in protein extracts from meningococcus is 38 kDa instead of 17 kDa (panel A). To gain information on the nature of this antibody staining we expressed ORF 206 in *E. coli* without the His-tag and including the predicted leader peptide. Western blot analysis on total protein extracts from *E. coli* expressing this native form of the 206 protein showed a recative band at a position of 38 kDa, as observed in meningococcus. We conclude that the 38 kDa band in panel B) is specific and that anti-206 antibodies, likely recognize a multimeric protein complex. In panel C is shown the FACS analysis, in panel D the bactericidal assay, and in panel E) the ELISA assay. Results show that 206 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 206 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots,

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antigenic index, and amphipatic regions of ORF 519 are provided in Figure 16. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 206 and the amino acid sequence encoded thereby is provided in Example 1.

### EXAMPLE 9

#### Expression of ORF 287

The primer described in Table 1 for ORF 287 was used to locate and clone ORF 287. The predicted gene 287 was cloned in pGex vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 287-GST fusion protein purification. Mice were immunized with the purified 287-GST and sera were used for FACS analysis (panel B), bactericidal assay (panel C), and ELISA assay (panel D). Results show that 287 is a surface-exposed protein. Symbols: M1, molecular weight marker. Arrow indicates the position of the main recombinant protein product (A). These experiments confirm that 287 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 287 are provided in Figure 17. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 287 and the amino acid sequence encoded thereby is provided in Example 1.

### EXAMPLE 10

#### Expression of ORF 406

The primer described in Table 1 for ORF 406 was used to locate and clone ORF 406. The predicted gene 406 was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 406-His fusion protein purification. Mice were immunized with the purified 406-His and sera were used for Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Results show that 406 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N.*



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*meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 406 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 406 are provided in Figure 18. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 406 and the amino acid sequence encoded thereby is provided in Example 1.

The foregoing examples are intended to illustrate but not to limit the invention.

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Claims

1. A method for identifying an amino acid sequence, comprising the step of searching for putative open reading frames or protein-coding sequences within one or more of *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.
2. A method according to claim 1, comprising the steps of searching a *N. meningitidis* nucleotide sequence for an initiation codon and searching the upstream sequence for an in-frame termination codon.
3. A method for producing a protein, comprising the step of expressing a protein comprising an amino-acid sequence identified according to any one of claims 1-2.
4. A method for identifying a protein in *N. meningitidis*, comprising the steps of producing a protein according to claim 3, producing an antibody which binds to the protein, and determining whether the antibody recognises a protein produced by *N. meningitidis*.
5. Nucleic acid comprising an open reading frame or protein-coding sequence identified by a method according to any one of claims 1-2.
6. A protein obtained by the method of claim 3.
7. Nucleic acid comprising one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.
8. Nucleic acid comprising a nucleotide sequence having greater than 50% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.

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9. Nucleic acid comprising a fragment of a nucleotide sequence selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.
10. Nucleic acid according to claim 9, wherein the fragment is unique to the genome of *N. meningitidis*.
11. Nucleic acid complementary to the nucleic acid of any one of claims 7-10.
12. A protein comprising an amino acid sequence encoded within one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.
13. A protein comprising an amino acid sequences having greater than 50% sequence identity to an amino acid sequence encoded within one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.
14. A protein comprising a fragment of an amino acid sequence encoded within one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.
15. Nucleic acid encoding a protein according to any one of claims 6-8.
16. A computer, a computer memory, a computer storage medium or a computer database containing the nucleotide sequence of a nucleic acid according to any one of claims 7-11.
17. A computer, a computer memory, a computer storage medium or a computer database containing one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.

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18. A polyclonal or monoclonal antibody which binds to a protein according to any one of claims 12-14 or 6.
19. A nucleic acid probe comprising nucleic acid according to any one of claims 5, 7-10, or 15.
20. An amplification primer comprising nucleic acid according to any one of claims 5, 7-10, or 15.
21. A composition comprising (a) nucleic acid according to any one of claims 5, 7-10, or 15; (b) protein according to any one of claims 12-14; and/or (c) an antibody according to claim 18.
22. The use of a composition according to claim 21 as a medicament or as a diagnostic reagent.
23. The use of a composition according to claim 21 in the manufacture of (a) a medicament for treating or preventing infection due to Neisserial bacteria and/or (b) a diagnostic reagent for detecting the presence of Neisserial bacteria or of antibodies raised against Neisserial bacteria.
24. A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 21.

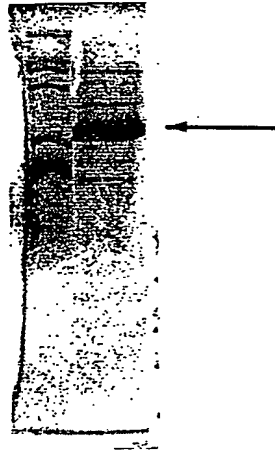
1/18

*FIG. 1A*

919 (46 kDa)

PURIFICATION

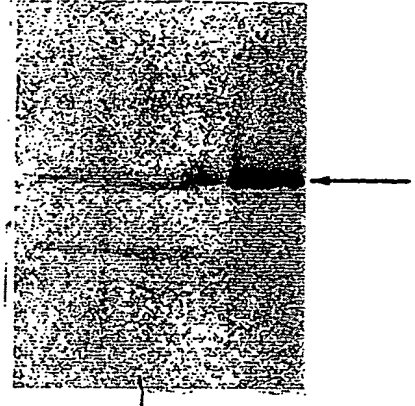
M1 919

*FIG. 1B*

919 (46 kDa)

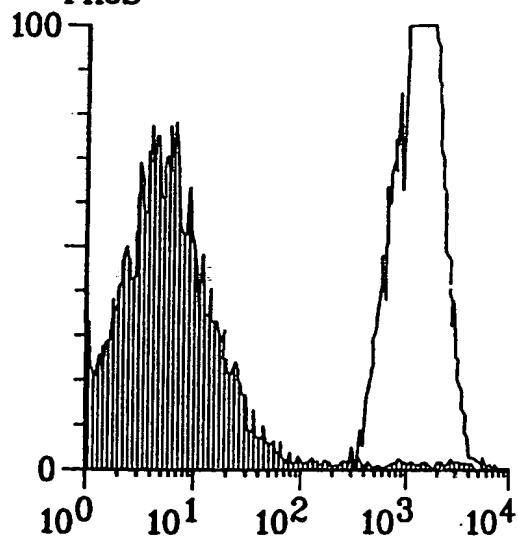
WESTERN BLOT

OMV TP PP

*FIG. 1C*

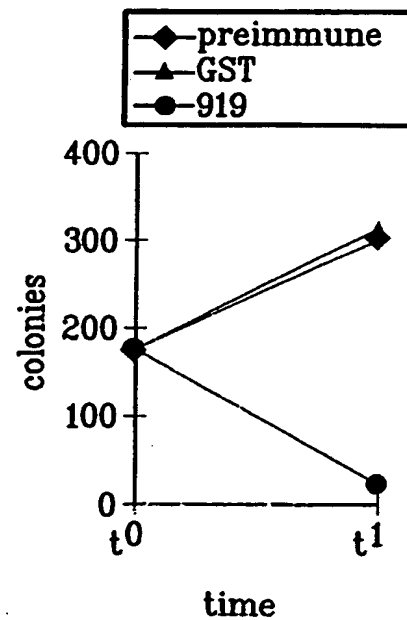
919 (46 kDa)

FACS

*FIG. 1D*

919 (46 kDa)

BACTERICIDAL ASSAY

*FIG. 1E*

919 (46 kDa)

ELISA assay: positive

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*FIG. 2A*

279 (10.5 kDa)

PURIFICATION

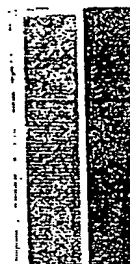
M1 279

*FIG. 2B*

279 (10.5 kDa)

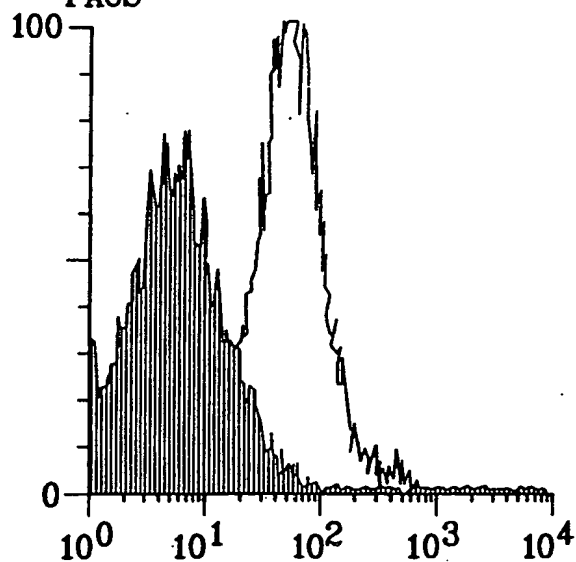
WESTERN BLOT

TP OMV

*FIG. 2C*

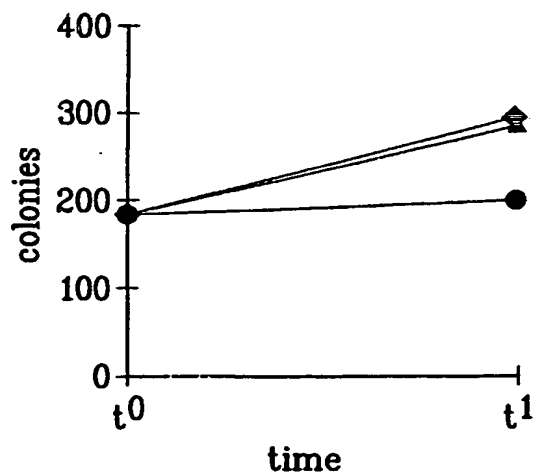
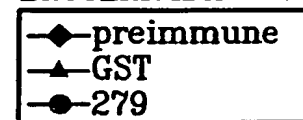
279 (10.5 kDa)

FACS

*FIG. 2D*

279 (10.5 kDa)

BACTERICIDAL ASSAY

*FIG. 2E*

279 (10.5 kDa)

ELISA assay: positive

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*FIG. 3A*

576 (27.8 kDa)

PURIFICATION

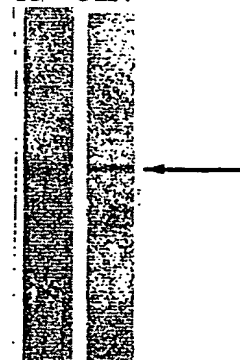
M1 576

*FIG. 3B*

576 (27.8 kDa)

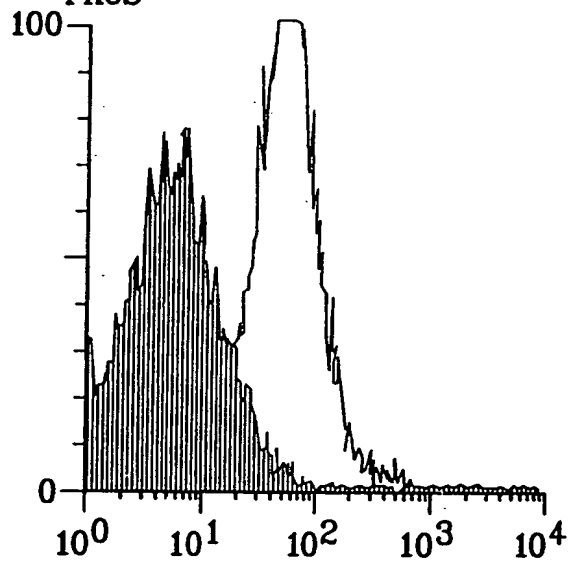
WESTERN BLOT

TP OMV

*FIG. 3C*

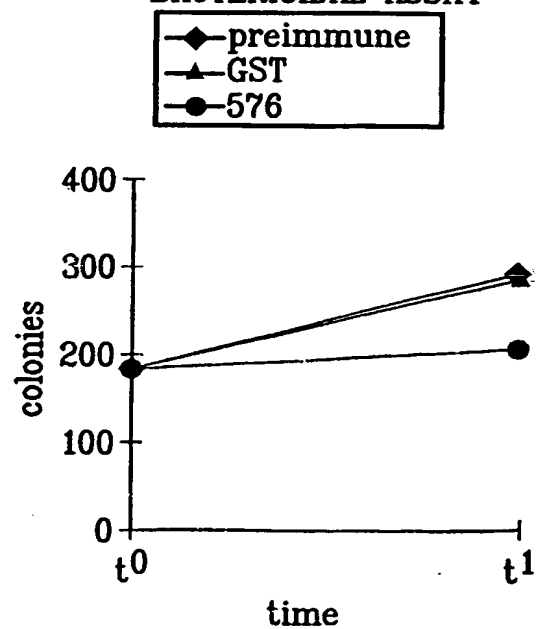
576 (27.8 kDa)

FACS

*FIG. 3D*

576 (27.8 kDa)

BACTERICIDAL ASSAY

*FIG. 3E*

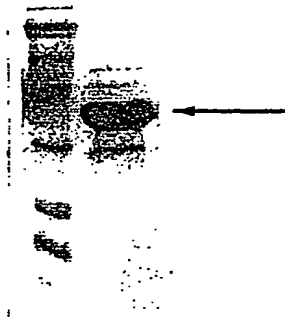
576 (27.8 kDa)

ELISA assay: positive

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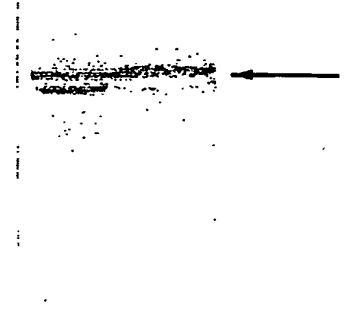
*FIG. 4A*

519 (33 kDa)  
PURIFICATION  
M1 519



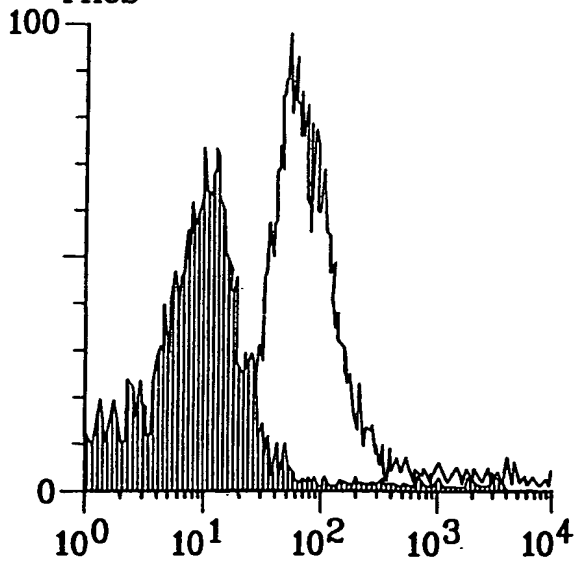
*FIG. 4B*

519 (33 kDa)  
WESTERN BLOT  
TP OMV



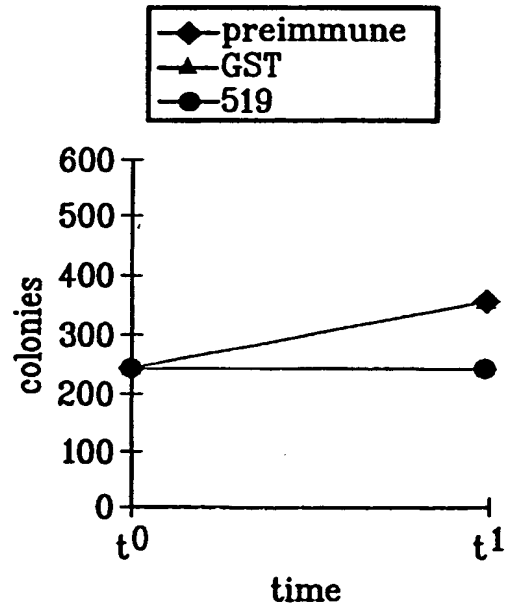
*FIG. 4C*

519 (33 kDa)  
FACS



*FIG. 4D*

519 (33 kDa)  
BACTERICIDAL ASSAY

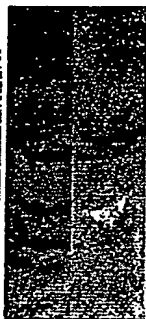
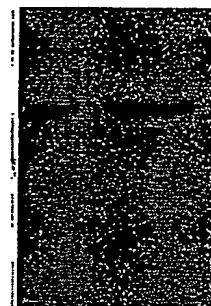
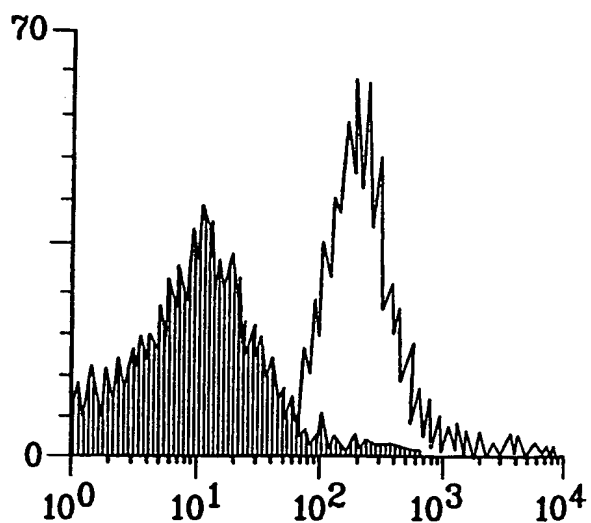
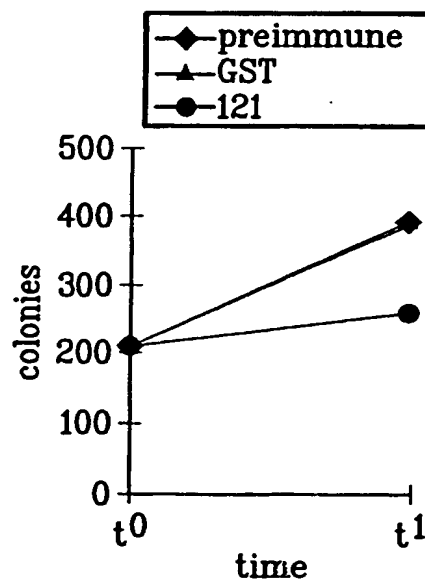


*FIG. 4E*

519 (33 kDa)  
ELISA assay: positive



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*FIG. 5A*121 (40 kDa)  
PURIFICATION  
M1 121*FIG. 5B*121 (40 kDa)  
WESTERN BLOT  
TP OMV*FIG. 5C*121 (40 kDa)  
FACS*FIG. 5D*121 (40 kDa)  
BACTERICIDAL ASSAY*FIG. 5E*

121 (40 kDa)

ELISA assay: positive

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*FIG. 6A*

128 (101 kDa)

PURIFICATION

M1 128

*FIG. 6B*

128 (101 kDa)

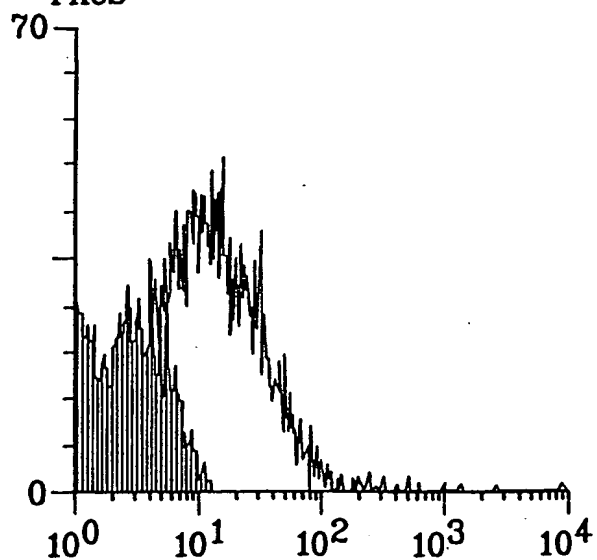
WESTERN BLOT

TP OMV

*FIG. 6C*

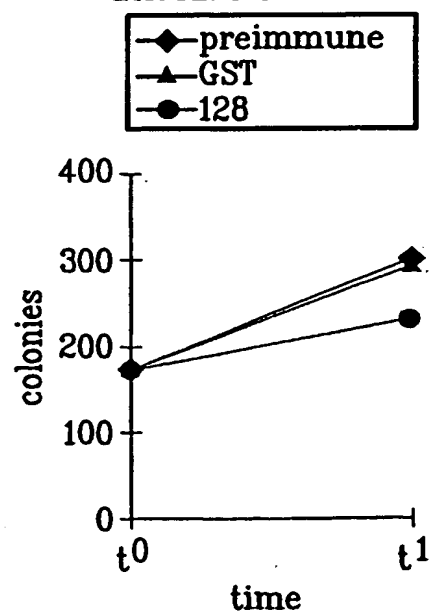
128 (101 kDa)

FACS

*FIG. 6D*

128 (101 kDa)

BACTERICIDAL ASSAY

*FIG. 6E*

128 (101 kDa)

ELISA assay: positive

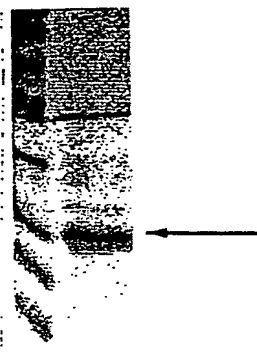
7/18

*FIG. 7A*

206 (17 kDa)

PURIFICATION

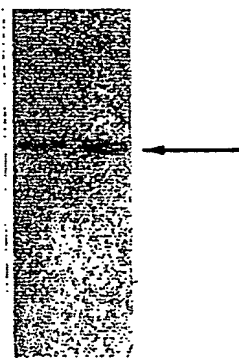
M1 206

*FIG. 7B*

206 (17 kDa)

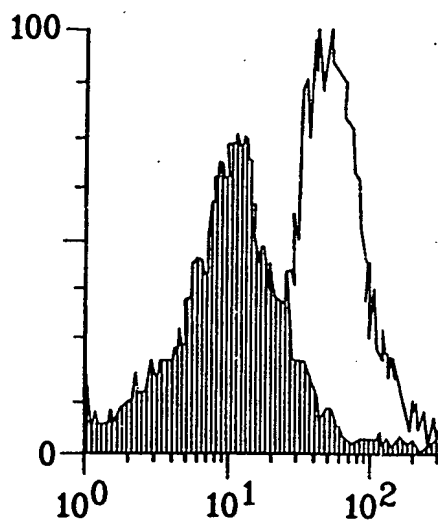
WESTERN BLOT

TP OMV

*FIG. 7C*

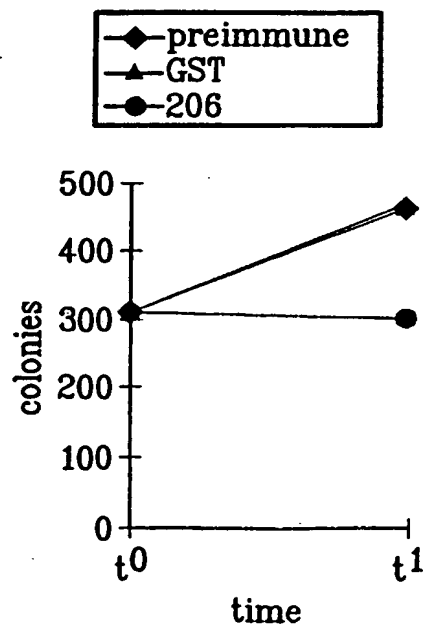
206 (17 kDa)

FACS

*FIG. 7D*

206 (17 kDa)

BACTERICIDAL ASSAY

*FIG. 7E*

206 (17 kDa)

ELISA assay: positive

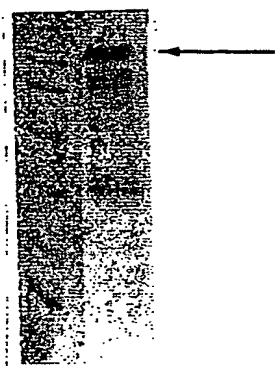
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*FIG. 8A*

287 (78 kDa)

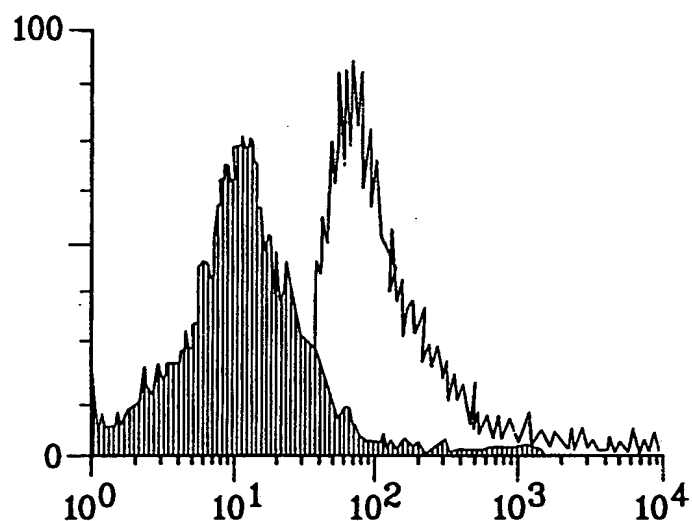
PURIFICATION

M1 287

*FIG. 8B*

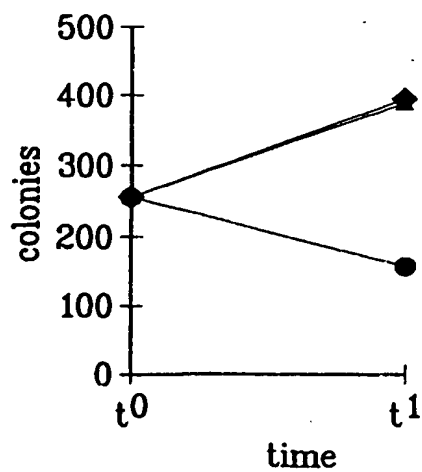
287 (78 kDa)

FACS

*FIG. 8C*

287 (78 kDa)

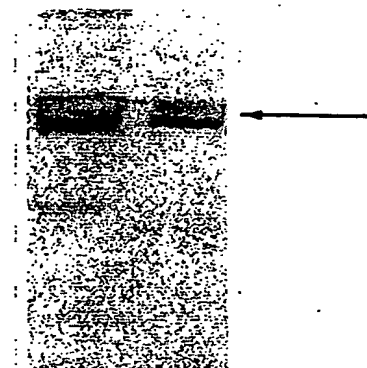
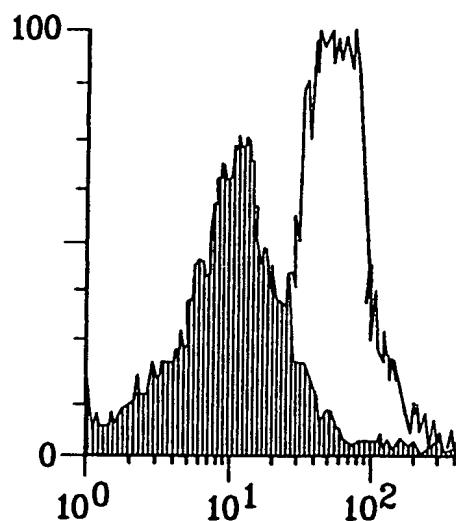
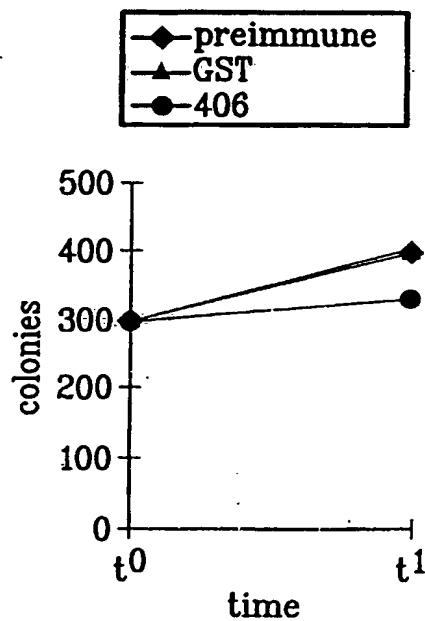
BACTERICIDAL ASSAY

*FIG. 8D*

287 (78 kDa)

ELISA assay: positive

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*FIG. 9A*406 (33 kDa)  
PURIFICATION  
MI 406*FIG. 9B*406 (33 kDa)  
WESTERN BLOT  
TP OMV*FIG. 9C*406 (33 kDa)  
FACS*FIG. 9D*406 (33 kDa)  
BACTERICIDAL ASSAY*FIG. 9E*406 (33 kDa)  
ELISA assay: positive

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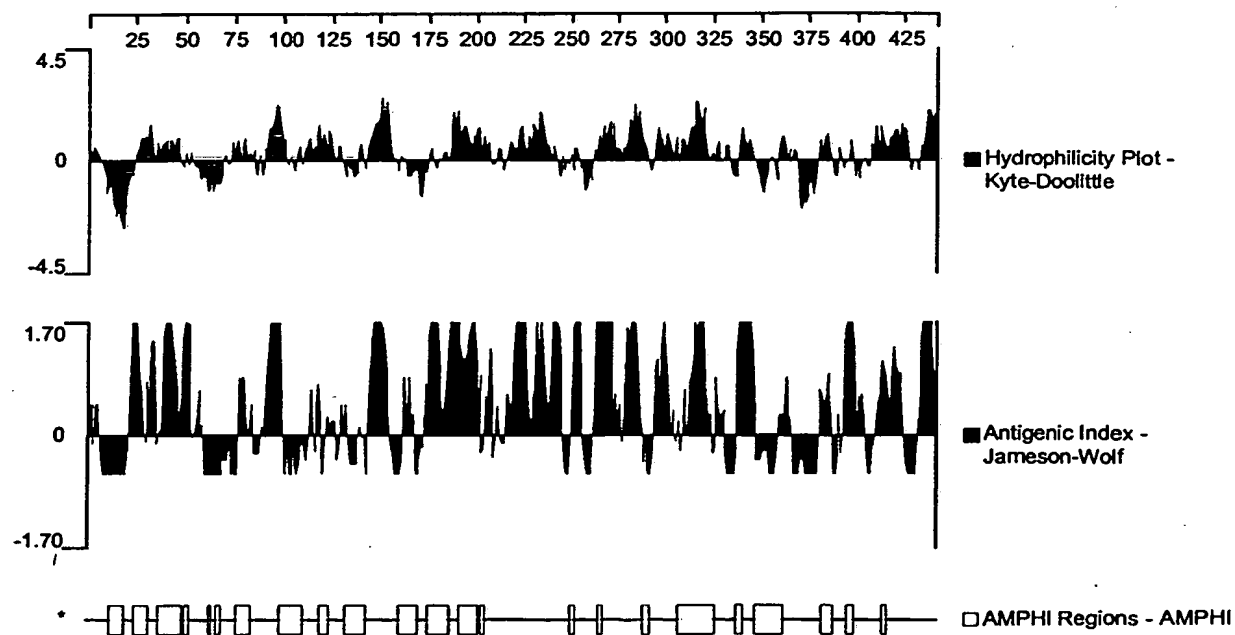
919**Hydrophilicity Plot, Antigenic Index and AMPHI Regions**

Fig. 10

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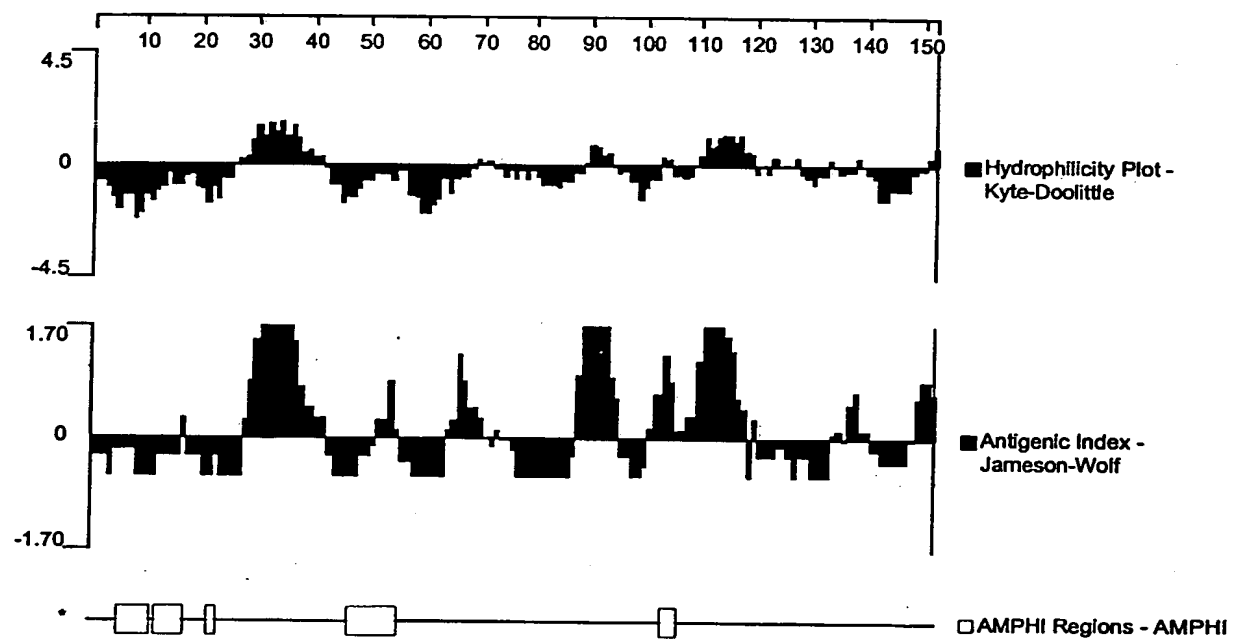
279Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 11

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576-1  
Hydrophilicity Plot, Antigenic Index and AMPHI Regions

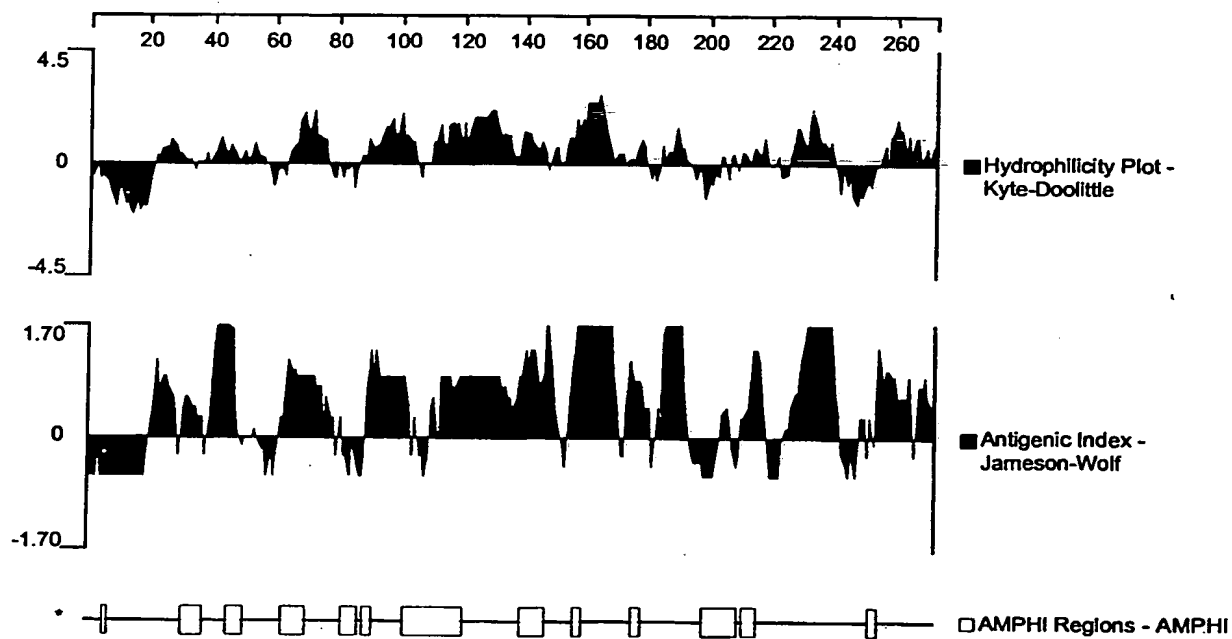


Fig. 12



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519-1  
Hydrophilicity Plot, Antigenic Index and AMPHI Regions

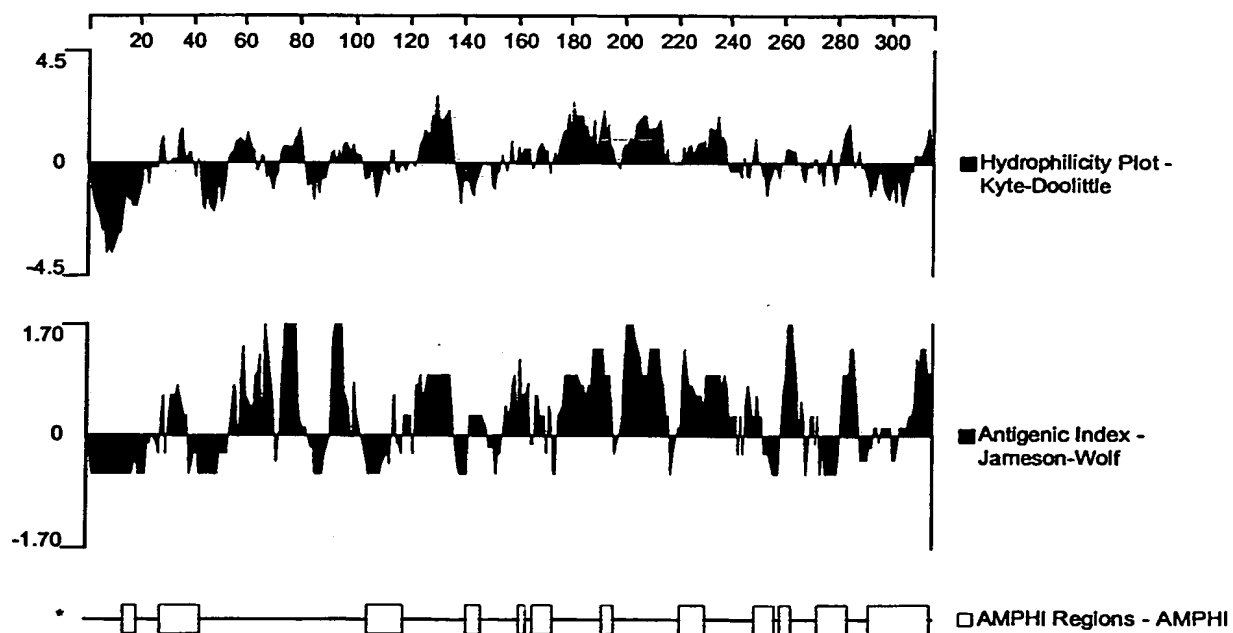


Fig. 13

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121-1  
Hydrophilicity Plot, Antigenic Index and AMPHI Regions

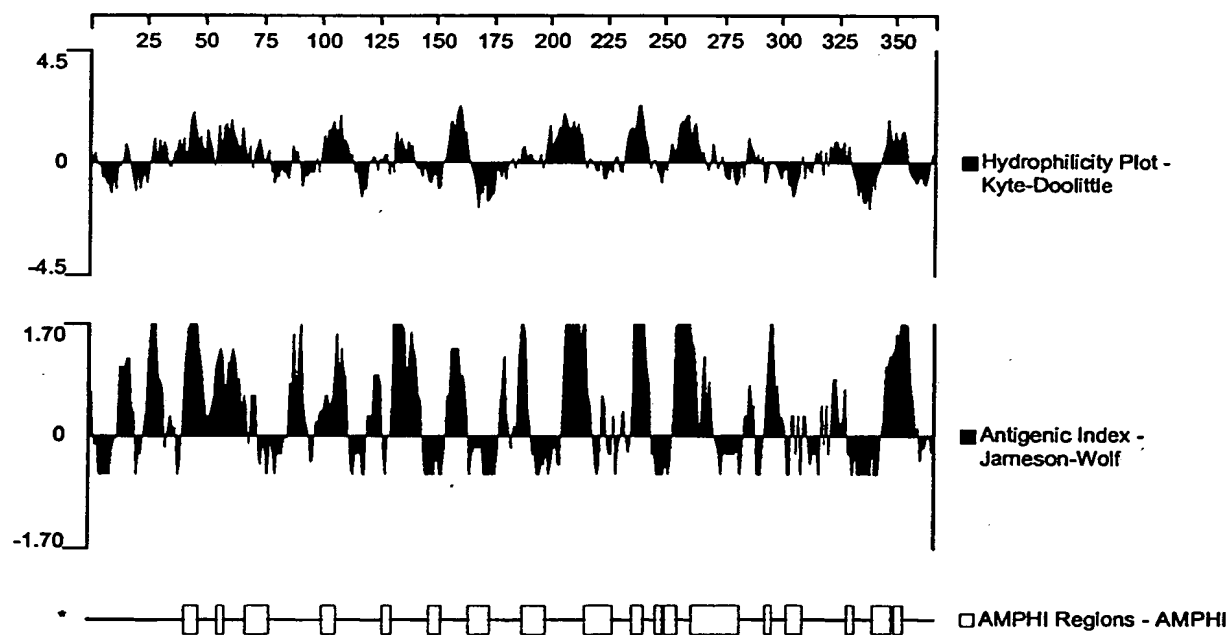


Fig. 14

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**128-1**  
**Hydrophilicity Plot, Antigenic Index and AMPHI Regions**

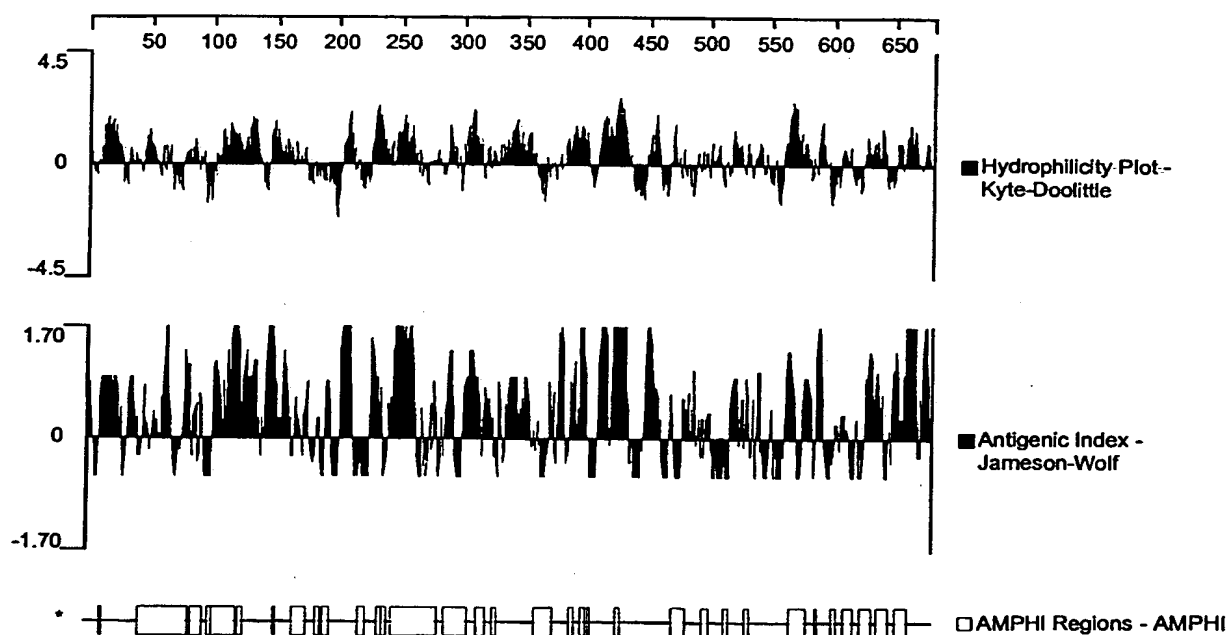


Fig. 15

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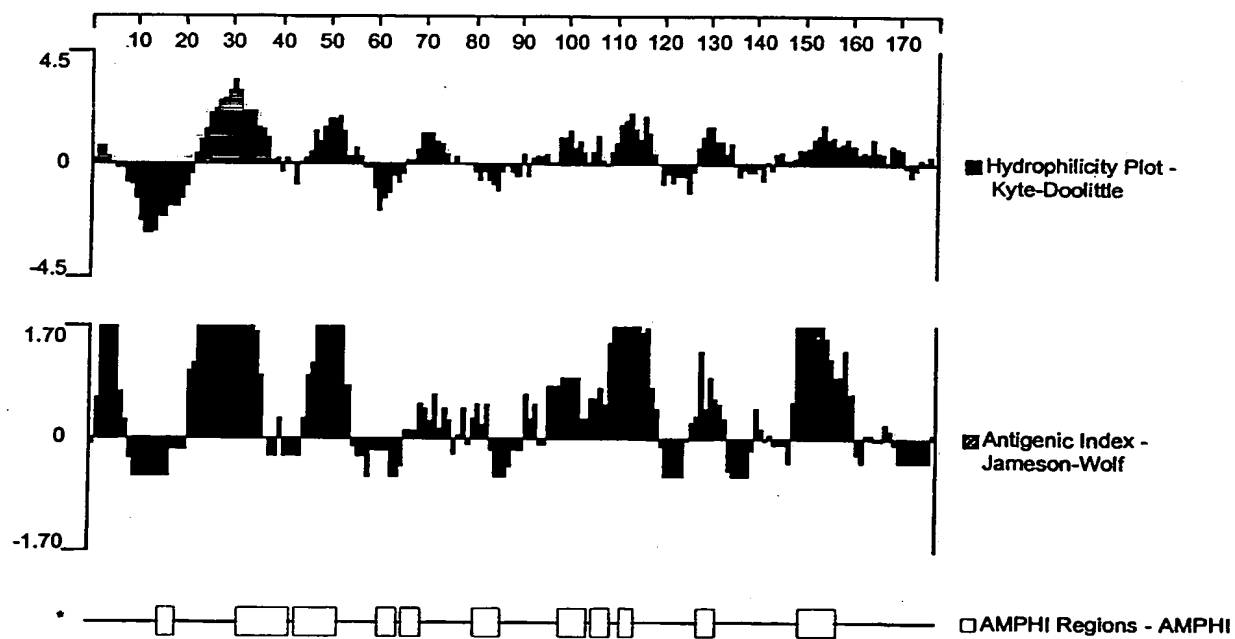
206Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 16

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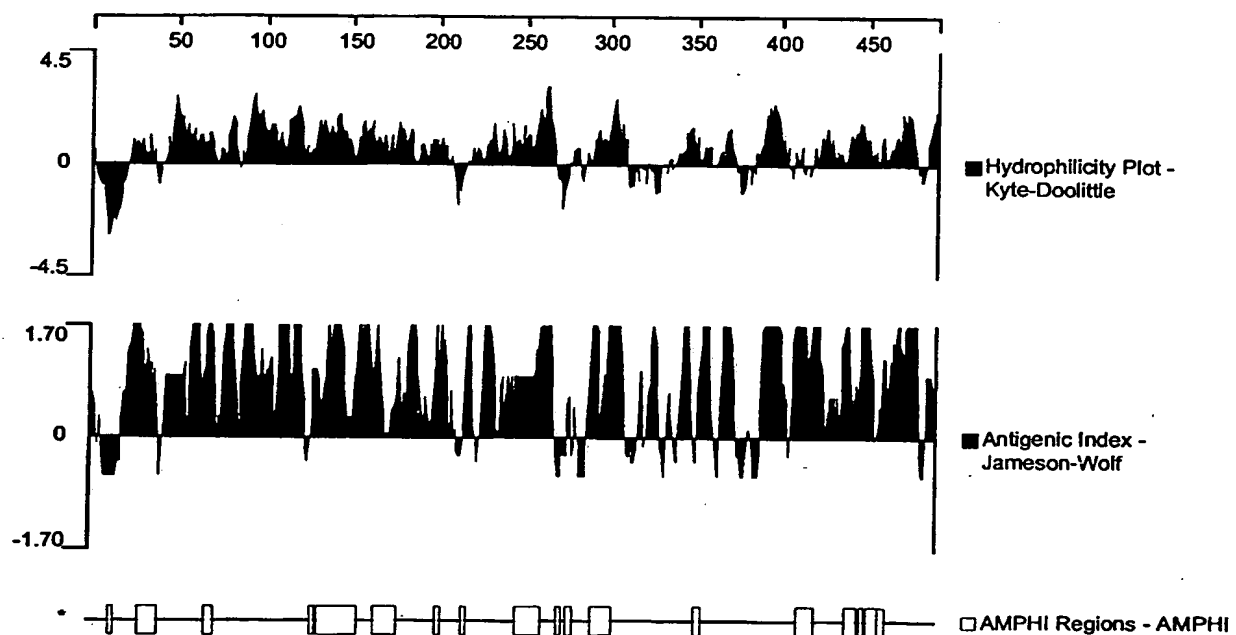
287Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 17

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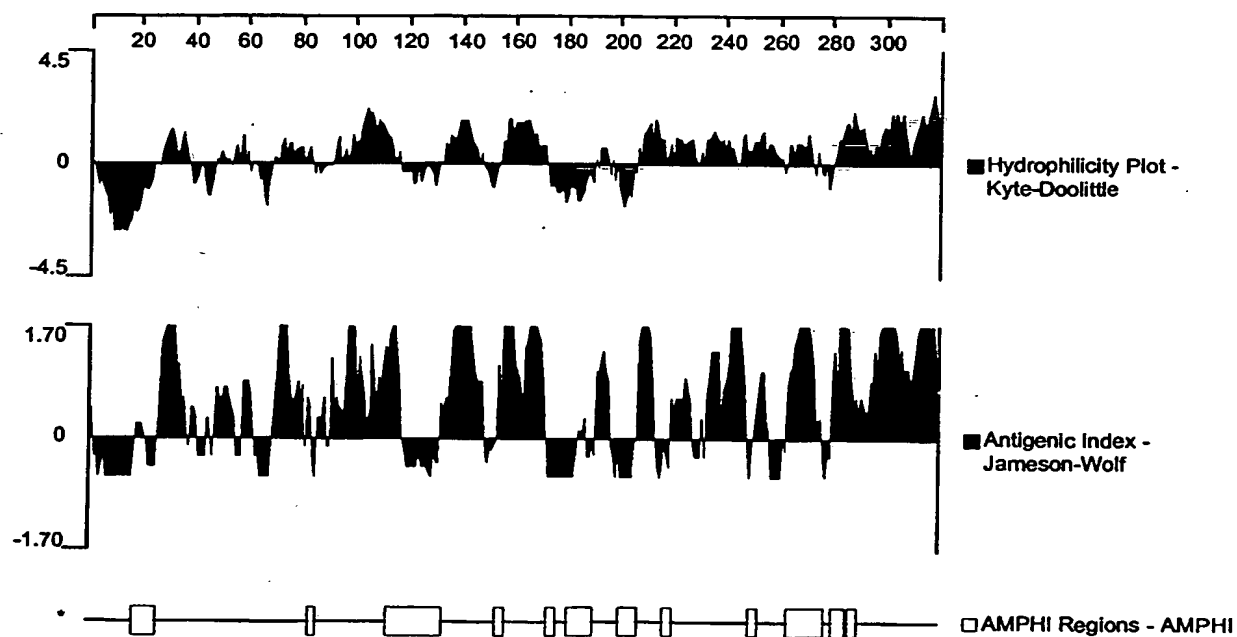
**Hydrophilicity Plot, Antigenic Index and AMPHI Regions**

Fig. 18

## Appendix A

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## APPENDIX A

The following DNA sequence was identified in *N. meningitidis* B <SEQ ID NO. 1>:

```
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ACCAACCTGCCGATATGCCCGGCTTTCAAGCCTGTAATGAAACAGTATTGTCCACAAAG
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```

## Appendix A

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## Appendix A

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## Appendix A

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## Appendix A

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## Appendix A

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## Appendix A

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## Appendix A

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## Appendix A

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## Appendix A

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## Appendix A

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## Appendix A

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## Appendix A

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## Appendix A

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## Appendix A

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TCACGTGTTTTTCATGCGCTCAAGAATTGTGATTGCTCATTTAGACGTTGCCCGCAT  
GGATCAGCCAGCAAAACAGTTTCTCCGTTAATACCGTTCAATACCGAAAAATGGTTGTTT  
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AAAGCATAACCTTGTGCTTCAAACCCAGTTCCGGCATTATGCGTTGATATCGTCAAAA  
GAAGCAGCATTTGGGTTTTATCCATTTGTCTAAGATTTCGGCTTCAGAAATATGTCTG  
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## Appendix A

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## Appendix A

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## Appendix A

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GAAGCCCGCGCCCATTTGCCCACTTGGCTGACAATCGCCGCTGCGTCAGCGCGCGCACC  
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CAAAGATTATCCGAAAGTTTGAAGTTCTAGATTCCCGTTTTTACGGGAATGACGGAATGT  
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GGAATCATGGGAATGACGGAATGTTGCGGGAATCATGGGAATGCGGGAATGTTTCGGTAA  
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CGACTTGGGCGATATGCTTGTGAAACGCGTTTTACGACCCCGTTACCGATACGGCGAA  
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TTGGCGGGCGATGGCGTGGAACGATGAAAACGCCGCGCGTTGCTGTGTCGGGACAATATT  
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## Appendix A

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[illegible]

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GGATCTAGACATTCAATGCTAAGGCAATTTATCGGGAATGACTGAACTCAAAAACTAG  
ATTCCCACTTTCTGGGAATGACGGGATATAGGTTTCCATGCGGACGCGTTTCGGATTAC  
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AGCTATTTAATAGTTACCTTTCTTATTTAAAAATAGCTTTCTCAAATTCATGAACGCC  
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TCAGCCTTCTTACAGAGTTGAAAACCGATGACTTTCAGTTTTGGGTTTGGCCGTAGT  
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GCCAAGTCATATACGGCTACTTTTCGGCTCTTGTGTGCGCTTGTCCATACCGAATGC  
CAAGCGGCTGCGCTCGCTGCTGATGATGCGTTTACGCTCAAAACCGGCGATACGGCC  
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TGCGGAATTTGAGGAGGACAGCTCTTTCCTTGTGCTTTTACCATGCGTTCGCGGTT  
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AAATTTGTGGCCGATCAACGTTTGGCGGCGTAAATAGTGTTTTGGCGTTGGTTACCGC  
TTGGCGTTTGGCAGGCGCACGAGGATTTCCGCGCGTCAAAATAAGCGATAACGGA  
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CAAAAGATGACCGGTAACATACGATACCTAAATAATCTCCATACCGATCCCTTCTT  
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GACGTTGATGGTGGCGGGTGGCTCCAGATGATACGATAACCAATTTGGCGGAGGTTT  
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CAAATTCGTTTCCAACAGATAAATATCCGAGGCTTGTGATGCTGCCGCTTTCAGA  
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AATCCACTAAGTCAATTCGGCGGCAATTTTTGTTGCTTTAACGGGATAGGCGGTTGGC  
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ACCCGACCGCTCCGCTCCGCTGAAACATCCAAACCAAGCGTATTGCCGCGCACGACGGTCT  
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TTCCCAATCGCCGATACGGGATTTCTGGTGCAGGATAGCGGGTTCGGTTTCTATGCCGAC  
ACGTTGGGTACTTTGCCCTTGGTCCATGTGCTGCCGTAGCGTTTGGGTAGGGTTTGGT  
GCATATTCTGAGATGTTGCCACAACGTGCTGCCGTTGCTTTTGTCTTGGCGAAGGTAGCG  
GTAAATGGTGTGTGGTGGAGCGTGTCTGGTGGTGTTCACAGGTAGGCGCATACTTG  
TTCGGGACTGAGTTTGGCGGGGATAAGGGTGTGATGTGCTGAATCAGCTGCGAATCGAG  
CTTATAGGGTTGTGCTTACGCTGTTTGTAGTCCGGCTTTGCCCGTGGGCTTTTTCGGC  
GCTGTATTGCTGCCCTTGGGTGCGGTGCCGCTGATTTCGCGGCTGATGGTGTCTTTGTG  
GCGGTTACGCTGTTTGGCGATTTCGGTGACGGTGCAGTGGCGGGACAGGTATTGGATGTG  
GTATCGTTGCCCTTTGGGTGCGGTGTAGCTCATGGCAATCTTTCTTGCAGGAAAGGT  
...CGTATGCTACCGCATACTGCGCTTTTCTGTAGGGAAGTTGCATTTCAATGCGAATC  
CGCCGTCGTCTGAAAAACATTATCTGCTGGACAAGAAAAACCTTGCCCGAACCATGTG

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[illegible]

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GCCGATAAAGGGTTTTTGGCCGGTTTGGTGCGGTTTCCATTATCGTCGTCATGCCGTCT  
GAAGAATAGTTGCCATCGTCTTCATCTTCATCTTCATCTTCATCGGGAGAGCCG  
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TTTCGGCAGACTGGGTATGCACCATCGCCGTTGCATCAGCGTTGGAAGCCGAGCGTG  
GGCCGGTTTTCGACCGTGTCTAAAAGGTGTTGCGGAATCAGGGGGTGTTCGCGCAACG  
CTGGCGGCGAGCTGTTCCGACACCACTTTCAAATCCGCGCGCAGAGCTTGAGGACGTTG  
TGGACGGAGGCATCTTCTCGATGAGTACCAAAAGCAGATGCTCGAGGCTGATAAATTCA  
TAATGAGCCTTACGCGCTCGCGGTAAAGCTGCTGCAAAATCTGTTCCAATTCGGGTGCA  
AGCATATTAAATCTCCTCGACAATACATTGCAGCGGATGCCCTTCGGCTTTTGGCCGCTG  
CATGACTTGTGTTGTTGTTGTTGGGCAATATCGCGCGTGAAGTGCCGACAGGCCTTT  
GCCTTCGTGATGAACCAAGAGCATTACCCTACCGCTGTTCTTGTCCGAGCATAAAGAT  
TTCGGTCAGGATTTCGACGACAAATCCATCGTGGTGAATCGTCGTTCAATAGGAAAC  
GCCGTAACGTTTCGCGCGCAGGGTGTTCAGACGGTGCAAGAGCGTGTGCGATTGGTGTG  
CGCGGTATAGTGTGTCCATTGAAAGCGCGGTTACAGCGCATTTTGTGTGATTTC  
GGTACTTTTGCCTATTTTCCCCTTTTGTAAACATAGCTTGACGTTTGTCTTAACAA  
ATGTAAAGAGCGGTTAAGCAGGATTGGGCATCCGCCGAGTATATCAAGCTGGAAGGAAC  
GCCAGACGGCTCGGTTTGGCGTATGCCCTGTTTGTGCTGATTGTTAATTTTGTAGTATA  
GGAAGTTTCTAATGGCAACCGGTATCGTAAATGGTTTAAACGACGCTAAAGGTTTGGTT  
TCATCAGCCTGATGAAGCGCGGAAGATTGTTGCTCACTTCTCAGCAATCAATATGG  
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AAGGCAAAACAGCGCCCAACATTCAGGCTGCTTAATTCCTGATGACGGTCAAATGTATA  
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GAAAACCCCAATCCCGCACGCTTATCCTGAATGTTGTGTACCTGTTGTGGACAAG  
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CGGATTGCGTTGTCGCTATGTTTCAAATACAGGTTGTGCGCGCGCGCGGCTTGGCCG  
TGGTCTGCATACCGAGTTTGGCGCGCTGTAAGTCTGAGGAAATAGCCTTCGACAATGC  
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TGCCGATGCTGTGATCAGGAACTGCTTTGGCGGTAGAGCGCGCCCGGAGAGTGGC  
CGACGAGGTGTCGATAAGACCGCCGAAACGGTGGTATCGAAGAGGACGGGTAGCTGC  
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GCAAAACCTGAACCGCGATTACCATTTTGAAGAAAGTGTGCTGCCGATATTTATTACAC  
GGTTACTGAAGAACATACCGCAAAATCCAAGCTGTTTGAAGAAATCTTATATACGCCCC  
CTTTTGTACTGATGCGCGGGCGGCTGCTGCGCTTGGCTGCGCGGCTGCGGTTG  
GGTCTGGATGCGCGCGGCAATGAACAGCAATGCGCTGTGAAAGCTTTCAGACGGCAT  
TTTAAGCACACGCACAGTAAACCCACGTTATGTGAGTGAAGTCAAACCGGATCC  
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CTTTGTGCTTCGCGCGGTGTGCAAAGTCTGCGCAATTGGACGACAAACTCGCTTCCCTC  
CTGCCCTTATCAATCGTTGACGAATTGCGAAGCCGCCGCCCGCTTTGGCGGATGCGGTT  
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GCCGTCGGTATGAGCGGTTTGGCGGCGATGGGGGCGAAAGTGGATTTCCTTGTGCCAAC  
CGCTTTGAACACGGCTACGGCTTAACGCCCGAACTTGCGGAAATCGCTGCCGCGCAGGGC  
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GGCAGCGTTGCCGATGTCGTCCCTCTCGACCACAACAACCGCATCCTCGTGTGCAAGGT  
TTGAACCGGATGCGCTCCGGCAAAATGCGCCCGGATCCGCGCCTTGTTTGAAGTGGCG  
CGGCGCGATTGGCGCAAGGCGCAGCCGTTTGATATGGGTTTGGCGTGGGCGCGCATC  
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GATTCCGAAGCTCAGGAACCTGGCGGCTCAGTTAAACAACCTCAATATCGAGCGCGCGAA  
ATCGAGCAGTCTATGCTCGAAGACGCACTGAATGATTCCCCGAAACCTGCGTTTCAGGT  
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TAGGCTTCCAGCATTTTTCACCAAGGATGCGCGCAACGCTTTCGCCGTTAAAGGTG  
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CCGATGCGGGTCAGGAACATTTTCATTGTTTCGGGCGTGGTGTTCGCGCTTCGTCGAGG  
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AGGCCTTTTCAATCAGCTTGGTTACACGGTCAAAGCCCATCAGGTCATAGAGGGCATCA  
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TTCTGCGCGGCTTCGACGCGTGGGCGCACTAAATGATGCGTTTCGACTTGGTGTTCCTC  
ATCGCATCGACGCGCGCGCAACGCGAGATAGGTTTGCCTGACCTGCCGCGCGGAGA  
CCGAATACGATGTCGTGGTTGAGCAGGGCGCGGATATAGCGGTTTTCGGTGGCGTCTG



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CCGCCGATGCTGCCGCGCTTGGTGCGGAAATAATAGGCGTGGTCATGGTTTTTTCTTGA  
TGACCGGCATCTTCGGTTTGGGCTTCGACGGCGCAAGCCTGATGTCGCCGTCGTTTAGG  
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GCGCGCTTGAAGTGAAATGTTCAAAACGGCGCTGATGTGGATATCGAGTGTTCGGCA  
AGTAAATCAAGTTGTGTCAAAAGAACCGCACAGACGCTGCAACGCCAAGTTGTTCGGT  
TCTTCTAAATGCAGGTGACGGTATGTGTATGAAGTCCGAATAGTTGGATATTGTG  
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AAGAGAACGATTCTCTAAGGTGCTGAAGCACCAAGTGAATCGGTTCCGTACTATTGTAC  
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TATCCGTTGCCGCTCTGAACAATAGATTCCGCACCGCGCAGCGTTTGCAATGACGGGC  
AATCCTTGGGACAGTGCTTCGATATAGACTACGCCGAAGTTTCTGTGCGGCTGGCAAGG  
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GCAGTGAGTAGGACATCGTGTCTTTGAGACGGCGAAGGTGCGAGACGGTCGAAACACG  
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TAAATTTAATCCACTATATTTTGAACCCGTAAGGGTGGTCAGGATGCTTGTGTTTTCT  
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TGCCAGTGCTGATTGTTGAAAAAATCCCGTCCACATCTGTTCCGATTGCGGATACCAT  
GAGGGGATGACGAGGACGTGCAAGGGTTCGGGATAGTGGGATTCGATATCGGAAGGCG  
GTTATTATAAGACAGACGCGACCGAATATTAAATGTTGCCCTACGCTAATGCAATTT  
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-GCTGCTGCGGGTAAACGCTCGGGGACGAGGTCGTCTGTAATTTGCTCTTTAAGCTCTTG  
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CAAAATATCGCGGATGACGCCGGCAGGCAGGACTTTTTCTTTTTCAGGGCGACGCG  
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GCGCGTACGACCGGCGAGCTATGCACGAGCGCGTCTTTGGTGCAGCTCAACCGGTTGAA  
TAAAGTAGATTGCGCGAGTTGGGGCGACCAACAGCGCGATGTTGGTTTCATGATTGA  
GTCTTTCTGTGTAAGTGCCTTTCGGGAGAACTGAACAGGAGCGAGGTGTCGCTTGGACAC  
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CAAATATTGATAAAGCGCGTCAGGAAAGCGGCTTCGAGGTTGGGATCGGACGGGGAGGG  
CTGCCATACGGTCTGTCTTTGCTTTGCGCGCGTACACTTCTTTCATGGCTTTGTGGGC  
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GCCGCGTTGGCGGCTACGGTAATCACGCCGTGTCGCCCGCAGAGCATGAACGGCAGGGC  
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TCATTTTTTCAGACGGCTGTTGTTAATAAGAACCGAAGACCTGTTAATGCCGACAAGGT  
CTCAACCTGTCTTACCGACGCGGTAACGCCAGGCTGCCAAAAAGGTTGGGAAATGT

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AAGCCAAACGCTACTTGGAAACCAACGGTAACGGAACACGTTTGGTGGCCCTTAACGCC  
CGGGCGTGATTACCGGGCGAGTGCAAAATCGGCATTATGCCGGCCACATCCATCTC  
CCGGCGCATCCGCTCATTTCCGTTCCGTTACATTGACTTACGAAGCGGTGGCACAAA  
CCACCAACTGGGCTTGGGTCAATCAACCTGTATCGGTATCGGCGCGACCCGATTCCGG  
GTATGAACCAATCGACGCACTGAACTTTTCAAGAAGACCGGATACCGACCGCATCA  
TCATGATCGGTGAAATTCGCGGTACTGCGGAAGAAGACGCGCAATACATCAATCCA

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ACGTAAGCAAACCTGTTGTCGGCTATATCGCCGGTGTACCGCACCTAAAGGCAAACGCA  
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